**NOAA Technical Memorandum NMFS**

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**JULY 1990**

# FIELD MANUAL FOR PHOCID NECROPSIES

# (SPECIFICALLY *Monachus schauinslandi*)

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**National Marine Fisheries Service**
William W. Fox, Jr., Assistant Administrator for Fisheries

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## INTRODUCTION

This necropsy manual is a guide for students and technicians in the examination of dead phocids, specifically the Hawaiian monk seal, *Monachus schauinslandi*. With this manual, the person performing the necropsy should be able to conduct a gross examination of a monk seal and collect the necessary tissues for microscopic pathology and toxicological studies. The necropsy instructions are based on the assumption that the seal's carcass is very fresh and time is not a limiting factor. However, because of variations in the condition of the carcass and the unpredictability of the time, location, and circumstances in which the carcass is found, it may be necessary to alter, abbreviate, or eliminate certain steps in the necropsy. When in doubt, always be more thorough rather than less, if time permits.

Appendixes A and B describe the specific techniques used for the collection and preservation of tissues, parasites, and other samples. A glossary also is included to clarify any terms unfamiliar to the reader.

## SUPPLIES

Before the necropsy is begun, the following supplies should be readily accessible to the individual performing the procedure.

1. Sharp knives (curved and/or straight 6 inch blades).
2. Scalpels and replaceable blades.
3. Small and large dissection scissors.
4. Long- and short-handled tweezers.
5. A sharpening steel.
6. Pruning shears (heavy enough to cut ribs).
7. Protective clothing (rubber boots; plastic aprons or 30 gallon capacity garbage bags with holes for head and arms).
8. Heavy-duty disposable rubber gloves.
9. Flexible and metal tape measures (in centimeters and at least 2.5 m long).
10. Plastic or wooden ruler (in centimeters and at least 15 cm).
11. Spring or platform weighing scale (10 kg capacity), preferably sensitive enough to weigh fetuses and small organs.

12. A 35 mm camera, 35 mm color film, macro-lens, and flash unit (ensure the batteries are working in the camera and flash unit).

13. Clipboard.

14. No. 2 pencils.

15. Necropsy forms.

16. Dissecting tray or board (ca. 0.5 x 0.5 m).

17. String.

18. Labeling supplies (waterproof Tyvek[1] tags; two permanent markers).

19. Containers for samples collected (Whirl-pak plastic bags are the best for small samples).

20. Fixatives [4 L 10% buffered formalin and 1 L alcohol-acetic-acid-formaldehyde-mixture (FAA); see Appendix A].

21. Alcohol (70% ethanol; see Appendix A).

22. Aluminum foil (for toxicological samples; see Appendix A).

## GENERAL SUGGESTIONS

### Precautionary Steps

Every effort should be made to avoid direct contact with a dead pinniped, because it may have had an infectious disease that is pathogenic in humans. Diseases such as "seal finger" have been contracted by contact with dead seals; therefore, the importance of protection should not be overlooked. Wearing protective clothing--including plastic aprons, rubber gloves, and rubber boots--greatly reduces the risk of exposure to contaminants when working in the field and in the laboratory. Upon completion of the necropsy, wash thoroughly with a disinfectant and either wash or dispose of any clothing that may have been contaminated during the necropsy.

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[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

### Photographs

Each photograph of the seal or tissues should include a scale reference (i.e., a ruler) and a label with the field number of the animal. Record each photograph and indicate the orientation of the shot (e.g., left front flipper, lateral aspect). This documentation is particularly important for lesions or internal structures since the orientation is often obscure in the photograph. A dark backdrop should be used for light-colored specimens to reduce glare and increase contrast.

### Descriptions of Abnormalities

Abnormalities should be described as precisely as possible, because formalin changes the color and texture of tissues and photographs can be unreliable. The standard observations to record on the necropsy form are relative size, shape, color, and texture of the abnormality.

### Collecting Abnormal Tissue

Each section of abnormal tissue collected during the necropsy should include some adjacent normal tissue (see Appendix A). The pathological changes in abnormal tissue can be so extensive that the tissue or organ of origin is not identifiable during microscopic examination in the laboratory; therefore, including some normal tissue from the periphery of the lesion will aid in the identification process.

### Labels

Labels are as important as the data, for an unlabeled sample may be completely worthless. With a permanent marker, the field number and nature of each sample collected should be recorded on a waterproof label. When the necropsy has been completed, verify that at least one label has been included with every sample. It is preferable to include two labels with each sample; double-bag the sample, placing one label with the sample and a second label in the outer bag.

## EXTERNAL EXAMINATION

Before commencing the necropsy, photograph all four sides of the seal (i.e., ventral, dorsal, and both lateral sides) and record on the necropsy form the location (i.e., the island or islet; the sector number) and condition (e.g., fresh, rotten, half-eaten) of the seal, as well as several other types of data: the animal's permanent or temporary field identification number, if applicable; whether the animal was found alive or dead, on the beach or in the water; a description of any obvious wounds; and any other relevant information that may be of significance.

Examine the carcass closely for lesions, swelling, pelage bleach marks, hind flipper tags, and tears where tags may have been. Record any damage to the carcass, including

that due to scavengers; describe and photograph all abnormalities, lesions, and scars (e.g., location, size, color). Check the condition of the umbilicus and record any discoloration or inflammation associated with the umbilical scar. The front and hind flippers should be manipulated to check for swelling, breaks, or deformities. Palpate the skull and rotate the head to check for skull fractures and cervical deformities or breaks; feeling any grinding or hearing any cracking and squeaking is abnormal. Open the animal's mouth to determine and record the color of the gums and tongue and, if applicable, broken teeth and any material blocking the pharynx. Inspect the anterior nares and nasopharynx for nasal mites, which, if present, should be collected and preserved in 70% ethanol after their number and location have been recorded on the necropsy form. Examine both eyes and record any abnormalities (e.g., bulging, dilated pupils). Finally, photograph and describe anything on the carcass that looks out of the ordinary, being as thorough as possible in recording all observations of the animal, for seemingly insignificant details may be important.

Figure 1 provides a diagram of the external features of male and female monk seals and the measurement references corresponding to the order in which the measurements are taken. If it is not feasible to measure standard length and axillary girth, an estimate should be obtained, if possible, and identified as such on the necropsy form by writing "est" after the measurement. An asterisk (*) is then placed next to the number, and the reason why the estimation is necessary should then be given under the measurement section. If an estimate is not possible, "ND" (not determined) should be indicated in the blank provided for the measurement, and the reason noted. Straight length is measured to the nearest centimeter with the seal on its ventral side. The seal is then turned over, and standard length is measured to the nearest centimeter with the seal on its dorsal side. The carcass should be as straight as possible. Manipulating the cervical vertebrae will ensure that the neck is extended as far as possible; however, no pressure should be applied to the neck as the measurement is taken. All length measurements are taken parallel to the body axis, from the tip of the nose to the tip of the tail. The axillary girth is measured to the nearest centimeter just posterior to the foreflippers. The tape is held securely (not tightly) around the torso for this measurement.

Next, the seal is sexed (Fig. 1). In male pinnipedia, the penal opening lies midventrally between the anus and the umbilicus. In female pinnipedia, the urogenital tract and anus open into a common furrow between the two hind flippers. The mammary teats are present on either side of the ventral midline, both anterior and just posterior to the umbilicus (Fig. 1). The umbilicus is in the center of the square area defined by the four nipples (most seal species have only two teats, corresponding to the posterior pair of teats in monk seals). The nipples are evident even in reproductively immature or inactive female monk seals. The condition of the mammaries can be determined by squeezing the teats: The presence of milk indicates the female was either in a very late stage of pregnancy or was postpartum at the time of death. Record the color and consistency (creamy or watery; clots or flakes present) of any milk exuded. These observations complete the external examination.

# INTERNAL EXAMINATION

## Opening

With the carcass lying on its back, make a straight incision through the blubber and underlying fat and muscle layers, starting from the anterior end of the lower jaw all the way back to the anus. This first incision should be made carefully, because the abdominal musculature is thin and the viscera may be sliced accidentally. If the blubber is thick, it is less of a concern, but caution is always advisable. Excluding the skin, measure to the nearest millimeter the thickness of the blubber on the ventral aspect of the seal, between the foreflippers. (Blubber is distinguishable from the underlying fat layer: blubber is whitish and has a dense consistency, whereas fat is looser, yellowish, and globular.)

With a scalpel, cut away the blubber and fat from either side of the ventral surface by starting at the front of the lower jaw and working backward to the tail (Fig. 2). From the abdominal region, collect two 100 g samples, one of blubber (excluding skin) and one of muscle, for toxicological assays. As muscle is sampled, care should be taken not to rupture the abdominal cavity.

A salivary gland, which is more "lobular" than lymph nodes and slightly less firm to the touch, can be found on each side of the head, at the angle of the jaw. Ventral to the exposed salivary gland are two joined lymph nodes (Fig. 3). Cut the muscles and connective tissue that hold the scapulae and flippers to the body and deflect the limbs back. Under the scapulae lie the subscapular lymph nodes. These nodes are typically about twice the size of a jelly bean and slightly flattened, but if an infection is present in the forelimb region, they can be swollen to several times their normal size. If they are enlarged, record this on the necropsy form and then fix a section of each node in 10% formalin.

## Thyroid

The thyroid consists of two separate lobes lying just posterior and lateral to the larynx and medial to the muscle layer on either side of the larynx and trachea (Figs. 3 and 4). To expose the thyroid, carefully cut away the muscle on the left side of the larynx and anterior trachea, then deflect the muscle dorsally to reveal the left lobe of the thyroid. Repeat the same procedure on the right side. Although the thyroid is somewhat darker than the surrounding muscle tissue, it is easily overlooked; examine the area closely. Palpating the area may reveal its location if examination of the tissue fails to do so. Once the thyroid lobes are located, carefully remove them with a scalpel. Weigh and measure the thyroid lobes and preserve both lobes in 10% formalin in separate containers labeled "right" and "left" accordingly, along with the rest of the identification.

## Abdominal Cavity

With a scalpel, cut through the ventral abdominal musculature from the diaphragm to the pubic symphysis and then carefully cut laterally to the posterior border of the diaphragm, separating the abdominal musculature from the diaphragm. Deflect the

musculature to either side and examine the abdomen in situ (Figs. 2 and 4). Describe (e.g., quantity, color, clarity) any large accumulation of fluid and sample it with a culturette if refrigeration is available (see Appendix B). Also check for any gross lesions or deformities (e.g., tumors, hemorrhages). Push the stomach and intestines away from the diaphragm to check for bulges in the diaphragm.

## Adrenal Glands

Usually the adrenal glands can be collected without much difficulty; however, they may have decomposed beyond recognition in very rotten specimens. To locate the adrenal glands, first push the intestines and stomach to one side and locate the kidneys (Figs. 5 and 6), which are beige to reddish brown. Kidneys in seals are lobulated, but their placement makes them easy to identify (Figs. 4 and 5). Immediately anterior to the left kidney is the left adrenal gland (Fig. 7), which is generally easier to locate than the longer and narrower right gland. To locate the right adrenal gland, first expose the right kidney and then follow the path of the vena cava anteriorly a few centimeters. The right adrenal gland is dorsal to the vena cava along a liver lobe (Figs. 5 and 6) and is located a few centimeters anterior to the left adrenal gland.

When an adrenal gland is located, hold it with a pair of tweezers and, with a scalpel, cut away the associated connective tissue and blood vessels. Trimming all excess tissue will ensure that an accurate adrenal weight (generally a few grams) is obtained. Weigh the adrenal gland once it is cleared of excess tissue. While holding the adrenal gland lengthwise between the forefinger and thumb, make a clean slice through its long axis. The incision should not bisect the adrenal gland and instead should extend about two-thirds of the way through it. However, an incision is not necessary if the animal is small and the adrenal gland will be examined histologically later.

After the incision has been made, examine the internal structure of the adrenal gland closely. The cortex constitutes the larger part of the organ and is cream colored; the medulla is at the core of the adrenal gland and is reddish. The color of the medulla should be relatively consistent throughout; darkened areas or streaks in the medulla should be recorded. The adrenal glands in a decomposing seal often will be uniformly deep red. In such cases, the cortex and medulla have decomposed to the extent that no abnormalities can be distinguished, but the glands should be collected anyway.

Both adrenal glands should then be fixed in 10% formalin for histology. Place them in separate containers labeled "left" and "right" accordingly and provide the rest of the identification.

## Female Reproductive Tract

As with other mammals, the ovaries of seals lie just caudal to the kidneys, which, as mentioned earlier, are along the dorsal wall of the abdominal cavity immediately posterior to the diaphragm (Fig. 4). The oval, pinkish-beige ovaries are attached to the dorsal wall of the abdominal cavity by the broad ligament. Each ovary is encased in a double fold of

peritoneum (the ovarian bursa), which can be many times the size of the ovary in a mature phocid (Figs. 8 and 9). The short oviduct leads to the uterine horn, which continues distinct after it is externally joined with the other uterine horn. The uterine horns eventually join caudally to form the "common" uterus (Figs. 8 and 9), which leads to the vagina via an opening in the cervix. The vagina passes through the pelvic girdle and ends as the vestibule in a common furrow with the anus. The bladder lies ventral to the vagina, and the urethra empties into the vestibule via a urinary papilla on the ventral surface of the tract (Fig. 8).

Before removing the reproductive tract, examine it in situ and record any obvious abnormalities or signs of pregnancy. Make an incision through the ovarian bursae and examine the ovaries superficially for corpora albicantia and a corpus luteum. Corpora albicantia are small, white scars lying just below the surface of the ovary; a corpus luteum is much larger than a corpus albicans, is yellowish-pink (in fresh specimens) and glandular, and may be somewhat convoluted. A mature female may have several corpora albicantia on each ovary, plus have a corpus luteum if pregnant or recently having ovulated. A corpus luteum in a pregnant female may dominate the ovary. If the animal is immature, the ovaries are smooth and ovoid.

Remove each ovary separately, count the number of corpora albicantia, and record that number on the necropsy form. Also record the presence or absence of a corpus luteum. Measure the diameter of the corpus luteum, if present, as well as the largest corpus albicans. Weigh and measure (length by width by diameter) both ovaries and record this information. Store both ovaries in 10% formalin in separate containers marked "right" and "left" accordingly and provide the rest of the identification. If the female is immature, the rest of the reproductive tract need not be examined as thoroughly as described below.

To remove the reproductive tract, first separate the pelvis at the pubic symphysis, forcing the two halves of the pelvis apart to make it easier to remove the tract. Sever the broad ligament (attached to the lateral borders of the horns, uterus, and vagina) and the bladder (positioned ventral to the tract) from the tract, plus any connective tissue or muscle holding the reproductive tract in place.

Once the urogenital tract is removed, lay it in a tray with its dorsal side up. With scissors, begin cutting at the dorsal aspect of the vagina and pause at the anterior end of the vagina (Figs. 8 and 9). Place a scissor tip into the lumen and cut open the cervix, which protrudes slightly into the vagina. Carefully continue cutting the length of the uterus and each horn; the lining is quite delicate. Extra care should be taken during this stage of the dissection, because a fetus may be present. If an embryo or fetus is found, record its length, weight, and, if possible, sex. If the fetus is fairly large, slice through its abdominal musculature to facilitate fixing its internal organs. Include a label with the fetus.

After the tract is cut open, spread it out and examine the lining carefully (Fig. 9) to see whether a scar from a recent pregnancy is present in one of the uterine horns. If a scar is found, record the horn in which it is located. The entire reproductive tract from sexually mature females should be preserved, if possible, in 10% formalin. If this is impractical,

preserve 0.5 cm sections of the uterine body and of the horns and, if present, a section of the scar in 10% formalin.

## Male Reproductive Tract

The testes are pinkish-beige oval bodies that lie inguinal, outside the abdominal musculature, but are covered by the skin and blubber. There is no external indication of their presence. To locate the testes, cut away the blubber lying ventral and lateral to the pelvis and then palpate the ventral and lateral borders of the pelvis for an ovoid body (the size varies considerably depending on the age of the seal and the time of year). The testes are situated just ventral and lateral to the pelvis, slightly posterior to the level of the knee joint (Figs. 10 and 11).

Once the testes have been located, carefully remove the thin musculature that covers them. The surface of each testis is covered by a fibrous sheath (the proper vaginal tunic). The epididymis lies along the dorsolateral border of the testis; the vas deferens extends from the tail of the epididymis to the base of the penis (Figs. 10 and 11). To remove the testis, sever the epididymis from the vas deferens. Check for the presence of sperm in the epididymis by making a few slices across the epididymis with a scalpel blade. If a milky substance is exuded, it can be assumed that sperm is present; record this finding and save a sample in an equal volume of 10% formalin.

After this procedure, separate the epididymis from the testis, but save and preserve a 0.5 cm section of the epididymis in 10% formalin for histology (the epididymis and testis from the same side may be stored in one container). Weigh each testis separately and measure its length, width, and diameter, without the epididymis, for the data record. Take a 0.5 cm cross section of each testis and place it in a container with the corresponding epididymis and 10% formalin. Label containers "right" and "left" accordingly, along with the rest of the identification.

## Kidneys

Pinnipeds possess reniculated kidneys. Each reniculus operates as a separate unit with its own cortex, medulla, and calyx. The reniculi are held together by connective tissue (Fig. 6). The kidneys are long and rather broad, surrounded by an outer cortex of connective tissue (the cortical capsule). They are not bean shaped as is characteristic of many mammalian kidneys. Examine the kidney in place, noting the presence of adhesions, abscesses, or hemorrhages. To remove the kidney, simply separate the organ from the dorsal musculature. Scrape the cortical capsule with a knife blade and reexamine closely.

Place the kidney on a flat surface and make several slices (a few centimeters apart) across the organ. Examine the sliced surfaces. The different regions of the reniculi should be visible in some of the exposed units. Record the location of any cysts, stones, or infarcts, if present. Collect a 0.5 cm section of the affected kidney and preserve it in 10% formalin

in addition to a sample of normal kidney tissue. Also collect a 100 g sample of kidney for toxicological assay.

### Spleen

The spleen in seals is a reddish-brown, flattened body with an irregularly notched margin and is considerably longer than it is wide (Fig. 12). It lies along the left margin of the stomach and is supported by the greater omentum and dorsal mesentery (Fig. 13). Remove the spleen, trim off excess tissue, and then weigh it and measure its length, width, and diameter. Gently palpate the spleen for lumps and cysts. Note if the organ is swollen; swelling may be due to blood engorgement. Make several slices in the spleen and record whether the cut surfaces are dry or "weepy"; check for hemorrhages and other discoloration. Any other abnormalities should be recorded as well. Collect and preserve a 0.5 cm section of the spleen in 10% formalin for histology.

### Pancreas

The pancreas in seals is a rather coarsely lobulated, yet compact, gland that is uniformly pinkish gray (Fig. 14) and is dorsal to the stomach (Figs. 4 and 13). To remove the pancreas, lift up the stomach and cut away the tissue connecting the pancreas to the stomach. Sever the tissue joining the pancreas to the liver and any other structures. Place the pancreas in a tray, palpate it for lumps, and examine it externally for discoloration (postmortem changes are fast, often discoloring the pancreas to a grayish tan). Make several incisions to check for internal abnormalities, which should be recorded if present. A 0.5 cm section of the abnormal tissue, as well as a sample of normal tissue, should be collected and preserved in 10% formalin.

Check for parasitic flukes (i.e., flat, oval trematodes) by cutting the main duct and several lesser ducts in the pancreas. Preserve any flukes in FAA and note their presence on the necropsy form.

### Stomach

The pinniped stomach is considered to be a curved dilation of the esophagus. Its single chamber consists of a sac with a pyloric region that is bent sharply backward on the body of the stomach (Figs. 2 and 13). The lacy, translucent tissue attached to the greater curvature of the stomach and reflected over the ventral surface of the intestines is called the greater omentum (Fig. 13). In a healthy seal, the omentum is fine, delicate tissue that drapes the anterior portion of the abdominal cavity. In certain diseased individuals, the omentum may become quite thickened and adhere to sites on the intestines, stomach wall, and/or abdominal wall. Photograph and record this condition if present.

To remove the stomach, first locate the junction between the esophagus and the stomach. With sturdy string, tie off the stomach at two locations: (1) near the base of the esophagus and just anterior to the diaphragm (Fig. 4) and (2) at the beginning of the intestines. Several centimeters away from the tied off points and on the sides away from

the stomach, cut through the esophagus and the small intestine. Remove the stomach, slicing through the diaphragm and any connective tissue holding it or the esophageal remnant in place. Place the stomach in a tray. With scissors, cut the string tying off the esophagus, then cut from the esophagus, through the stomach wall, to the pyloric sphincter. Examine the stomach lining closely for otoliths, squid beaks, longusta shells, crustacea, nematodes, and abnormalities (e.g., ulcers and hemorrhages; Fig. 15). Collect all nematodes found in the stomach and preserve them in FAA. Weigh the stomach contents and record the weight as well as the nature of the contents. Stomach contents (except nematodes) should be preserved in 70% ethanol or frozen for later examination. Record any abnormalities found in or on the stomach and collect a sample of the affected tissue for histology. It is not necessary to collect a sample of normal stomach tissue unless abnormalities are present in the organ as well. Sever the esophageal remnant and the intestine at the stomach proper, then weigh the empty stomach.

If dissecting the stomach in the field is impractical, fill the stomach with fixative and return it to the laboratory for examination. To prepare the stomach for fixing, first tie off the stomach where it meets the intestines; make sure it is tied securely so no leakage can occur. Fill the stomach with 10% formalin and then tie off the stomach where it meets the esophagus.

## Intestines

The intestines decompose very rapidly, so they may be very flaccid, discolored, and perhaps distended with gas (Figs. 2 and 16). If this is the case or if time is limited, select and cut a segment of the gut, tie one end with string, fill with 20% formalin, and then tie off the other end. However, if the specimen is fresh (i.e., the small intestine is still round and firm), using the following procedure to examine the intestines will provide valuable information.

Beginning at the mesentery, cut the intestines just enough to allow them to straighten. The mesenteric lymph nodes will be examined later (see Mesenteric Lymph Nodes below); therefore, care should be taken to avoid damaging them when cutting the mesentery away from the intestines (Fig. 16). Beginning at the anterior end of the small intestine, examine the external surface and record any obvious lumps, perforations, hemorrhages, and other abnormalities. Place the intestines in a tray and, with scissors, cut open the gut lengthwise. Examine the lining for lesions; excise and preserve samples of any significant lesions in 10% formalin. Record the presence of parasitic worms (tape and thorny headed worms) and preserve any intestinal parasites in FAA.

## Mesenteric Lymph Nodes

Lying in the mesentery are a couple of lymph nodes known as the mesenteric lymph nodes (Fig. 16), which are the largest lymph nodes of the abdomen and are therefore the easiest to locate and examine. (The largest of the nodes is sometimes confused with the pancreas.) After locating the mesenteric lymph nodes, make a few incisions through each node. The nodes consist of a lighter colored cortex and a darker colored medulla. The

nodes may be congested (i.e., dark red or brown speckles are present) or swollen. Record the condition of the lymph node, noting the degree of congestion and the placement of any dark spots. Collect a 0.5 cm section of the mesenteric lymph node and preserve it in 10% formalin for histology.

## Liver

The last organ in the abdominal cavity to be examined is the liver, which is also the largest internal organ of the mammalian body. In pinnipeds, the liver is usually deeply divided into five to eight lobes that surround the hepatic sinus. The liver in seals is more elongated and its lobes have a more pointed shape (Figs. 2 and 4) compared with the liver of other carnivora. The liver is located at the anterior end of the abdominal cavity, immediately posterior to the diaphragm and ventral to the stomach (Figs. 2 and 4). It is dark red in fresh specimens; however, it may be brown and/or greenish with a metallic sheen in more decomposed specimens.

To remove the liver from the body cavity, simply cut the fascia and blood vessels that join the liver to the diaphragm. Place the liver in a tray and inspect it for rounded margins of the lobes and for any lumps, white spots, and cysts. After examining the liver externally, make several incisions (one every few centimeters) across the lobes. Use a sharp knife or scalpel to slice open any lumps. Examine each incision and any lumps for the presence of cysts and parasitic flukes (i.e., flat, oval trematodes). Record the presence of any cysts and flukes; preserve any flukes in FAA. Collect and preserve a 0.5 cm section of normal as well as any abnormal liver tissue and preserve in 10% formalin for histology. Weigh the liver and collect a 100 g sample of liver for toxicological assays.

Examine the gall bladder, located in a depression on the ventral aspect of the liver (Fig. 2). Note the color of the bile.

## Thoracic Cavity

To examine the thoracic cavity, trim any excess muscle tissue from the ventral surface of the rib cage. Pull the anterior end of the sternum while cutting the ribs at the junctures of the true ribs and the costal cartilage, the costochondral junction (Fig. 17). This junction is found just lateral to the sharpest curve of the ventral surface of the rib cage. There is a slight bulge at the site of this junction. In immature seals, the costal cartilage is cartilage; in older individuals, it is calcified. The costochondral junction may be hard to locate in adults; in which case, pruning shears can be used to cut through the ribs.

After the ribs are cut down one side, push the sternum to the opposite side and examine the thoracic cavity (Fig. 18). Move the lung around to check the pleura separating the two lungs. The pleura should be thin and transparent. Note any abnormalities, such as excessive thickness or white coloration. Look for tags of fibrous material between the lung and the pleura or between the pleura and the rib cage; tags can be thin or very hard and lumpy. Also make a note of any fluid, unusual in color, amount, or consistency. Examine the diaphragm, which is located at the posterior end of the thorax. Note if the pleura or

diaphragm are perforated. The heart lies between the lungs and is encased in the pericardium.

Cut down the other side of the rib cage. Cut away the diaphragm and pericardium where they are attached to the sternum and place the sternum with joined costal cartilage to one side (Fig. 19). Be careful not to slice open the pericardium when separating it from the sternum, because it will be examined more closely when the pluck is removed. The sternal unit can be used in place of a tray, if necessary.

### Thymus

The thymus--a light gray, distinctly lobulated organ with a pink tinge in fresh specimens (Fig. 20)--is laterally compressed and lies in the anterior ventral portion of the thoracic cavity (Fig. 4). Predominantly lymphoid in nature, the thymus is largest in very young phocids, but even in old individuals, it may not be completely atrophied. Considerably smaller in subadult and adult phocids, the thymus can be difficult to locate. Once it is located, trim away any connective tissue holding the thymus in place and, if possible, weigh it. Because of the diffuse nature of this organ, obtaining an accurate weight is not always feasible. Collect and preserve a 0.5 cm section of thymus in 10% formalin for histology.

### Removing the Pluck

Removing the heart, trachea, and lungs together (i.e., the pluck) from the thoracic cavity reduces the risk of damaging the atria. The first step is to excise the tongue by cutting through the symphysis joining the two halves of the lower jaw and then cutting along either side of the tongue. Grasp the tongue and pull it caudally while cutting the connective tissue that attaches the pluck to the dorsal and lateral walls of the thoracic cavity. The esophagus can be left joined with the trachea, since it will not interfere with dissecting either the lungs or the heart. Next, remove the pluck from the thorax by cutting through the aorta, as well as the caudal vena cava and a small amount of connective tissue posterior to the pluck. Lay the pluck, ventral side up, in a tray for further examination (Fig. 20). The lungs and the heart can be examined and dissected while still associated.

### Heart

The heart is encased in the pericardium, a thin, translucent, fibrous tissue sac (Figs. 18 and 19). Cut away the pericardium to expose the heart and note the amount and color of the pericardial fluid. Examine the placement of the pulmonary artery, aorta, and pulmonary vessels of the heart with the ventral side up (Figs. 20 and 21). Note any external infarcts, hemorrhages, or other abnormalities; collect a 0.5 cm section of tissue from such areas and preserve in 10% formalin for histology.

Carefully cut from the ventricles through the atria (Fig. 21). Note the nature of any clots or their absence. Remove the clots and examine them for heartworms. Collect any heartworms found, preserve them in FAA, and note their presence on the necropsy form.

Examine the internal structure of the heart. The papillary muscles, present on the walls of the ventricles, control the valve cusps by thin, strong tendons (Fig. 21). The valve cusps are normally thin and transparent. If the cusps are noticeably thickened or have become detached from the wall, make a note of this. The walls of the atria should be thin, almost translucent, with muscle bands forming an interlacing network on the walls of both atria (Fig. 21). Any thickening of the walls should be recorded and a 0.5 cm thick section collected for histology. Examine the grooves between the muscle bands for clots resembling chicken fat; note any clots that are tightly adhered to the atrial wall. Small, stringy attachments are a common postmortem reaction.

In fetal or stillborn phocids, determine whether the foramen ovale is still open, by probing the wall between the atria. Note the opening between the atria if it is present.

Continue cutting the pulmonary artery and the aorta to check for plaque, scarring, and heartworms. Plaque appears as raised, firm scars on the wall of the artery. Collect and preserve a 0.5 cm thick section of plaque in 10% formalin; preserve the worms in FAA.

Prepare the heart for weighing by cutting the great veins at their point of entry and by cutting across the pulmonary artery and aorta at the top of the semilunar valves. After weighing the heart, collect and preserve a 0.5 cm section of heart ventricular tissue in 10% formalin for histology.

## Lungs

Examine the lungs superficially and note their color and consistency (Fig. 20). They should be light pink and spongy: record any blotching, especially congested areas (deep red blotches; see Fig. 19). Palpate the lungs between thumb and fingers to locate firm or granular areas. Section any such areas to check for abscesses, tumors, or tubercular lesions (small fibrotic granules that, when sectioned, will appear as white, glistening spots within the tissue). Collect and preserve a sample of the affected tissue as well as of the apparently normal lung tissue.

Starting at the anterior end, use scissors to cut from the trachea to the bronchi. Following the air pathways, proceed to cut open each lung. Squeeze the lung tissue surrounding the bronchi that have been cut open. Note the presence of copious amounts of froth and fluid. Check for the presence of parasitic worms as well as any obstructions or growths not visible externally. Collect any worms and preserve them in FAA; record their presence on the necropsy form.

Occasionally, stomach contents and worms are aspirated in the throes of death. Therefore, worms found in the trachea or bronchi are not to be confused with lung worms. However, their presence should be noted, and a sample of the worms collected. Any abnormalities found internally should be recorded, and a 0.5 cm thick sample collected and preserved in 10% formalin. Also, collect a sample of normal tissue from each lung for histology.

Before weighing the lungs, cut off the bronchi at their point of entry to the lungs. Weigh each lung separately.

### Removing the Skull

The last step in the necropsy is to remove the skull so that it may be brought back to the laboratory. Clear away muscle tissue from the posterior end of the skull and the anterior cervical vertebrae. Separate the head from the body by locating the junction of the head and neck (Figs. 3 and 4). This is done by palpating the posterior end of the skull on its ventral and lateral aspect; the cervical vertebrae are slightly narrower than the skull. Place the tip of the knife blade between the skull and the first vertebrae, then rotate the blade to weaken the joint. Manipulate the head in addition to carefully twisting the blade. With a little effort, the head will begin to separate from the cervical vertebrae.

Once the head is disarticulated from the neck, remove as much tissue as possible from the skull, including the eyeballs. Next, if the animal is very fresh, remove a brain sample for histology. Use a narrow spatula or scalpel to collect a sample through the foramen magnum. Collect a 100 g sample, if possible, for toxicological assays using the same technique. Once the samples are extracted, gently scrape out the rest of the brain and set the skull in a place where it can dry thoroughly before being returned to the laboratory. This procedure completes the necropsy.

## ACKNOWLEDGMENTS

I thank the New England Aquarium (NEA) for granting me access to their laboratory facilities. I particularly thank Greg Early, NEA, for providing me with the *Phoca vitulina* specimens, which I used to produce this manual, as well as for sharing his considerable knowledge of phocid anatomy and pathology. I also thank William G. Gilmartin for providing me with the opportunity to produce this manual as well as for sending me the two *Monachus schauinslandi* specimens used in this manual. Reviewers Robert A. Morris and Thomas R. Sawa provided valuable input. I appreciate the editorial and technical assistance provided by Thea Johanos and Leslie Williams.

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# Figures

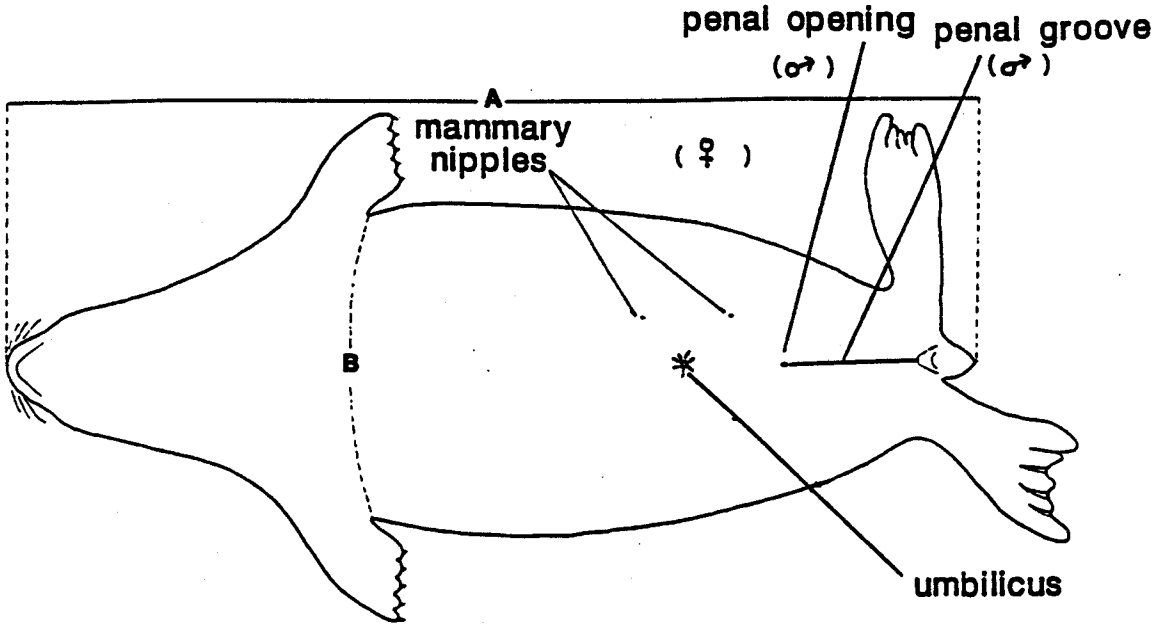


Figure 1.--The external features of male and female *Monachus schauinslandi*, and the measurement references corresponding to the order in which the measurements should be taken. (A) Straight length (when seal is lying ventrally) and standard length (when seal is lying dorsally). (B) Axillary girth.

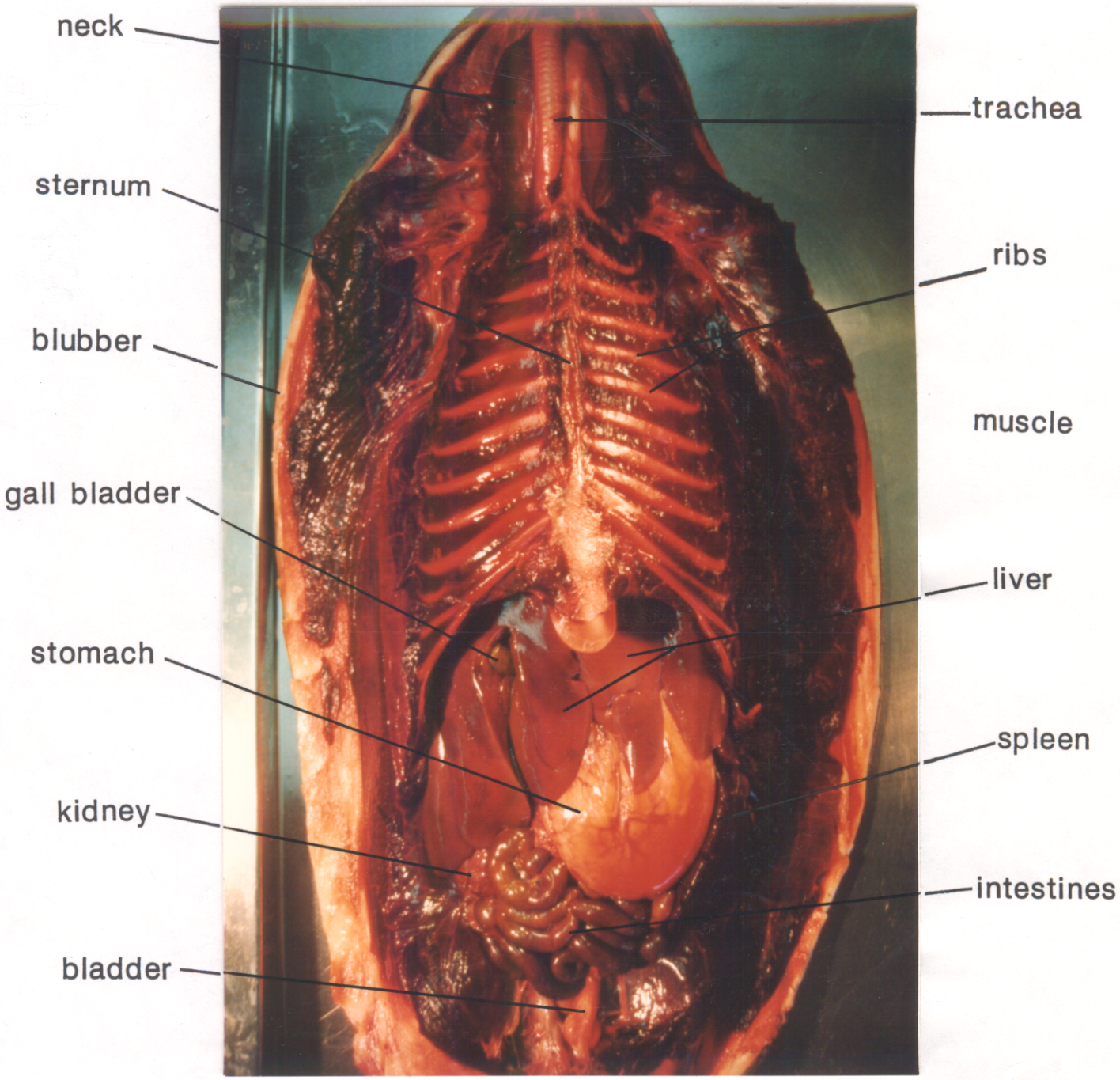


Figure 2.--Ventral aspect of a *Phoca vitulina* with blubber and muscle layers removed to reveal the trachea, thorax, and abdominal cavity.

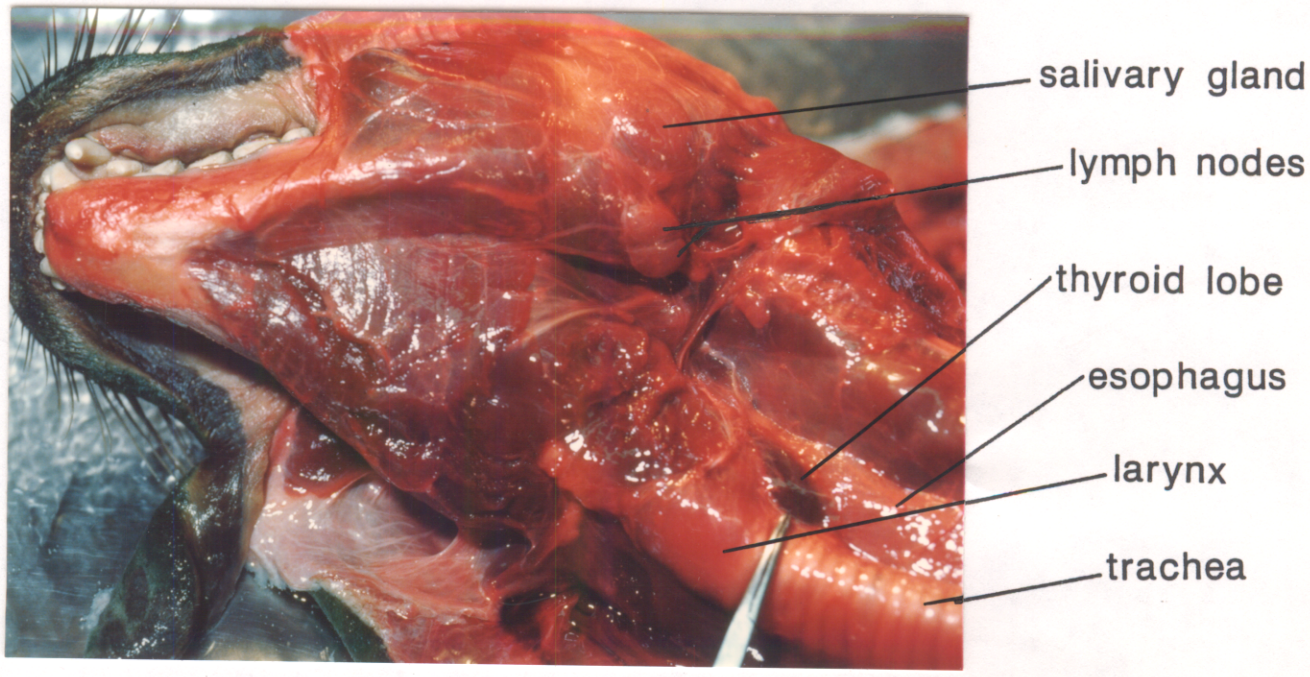


Figure 3.--Left lobe of the thyroid gland in a *Monachus schauinslandi* revealed; salivary gland and lymph nodes also are exposed.

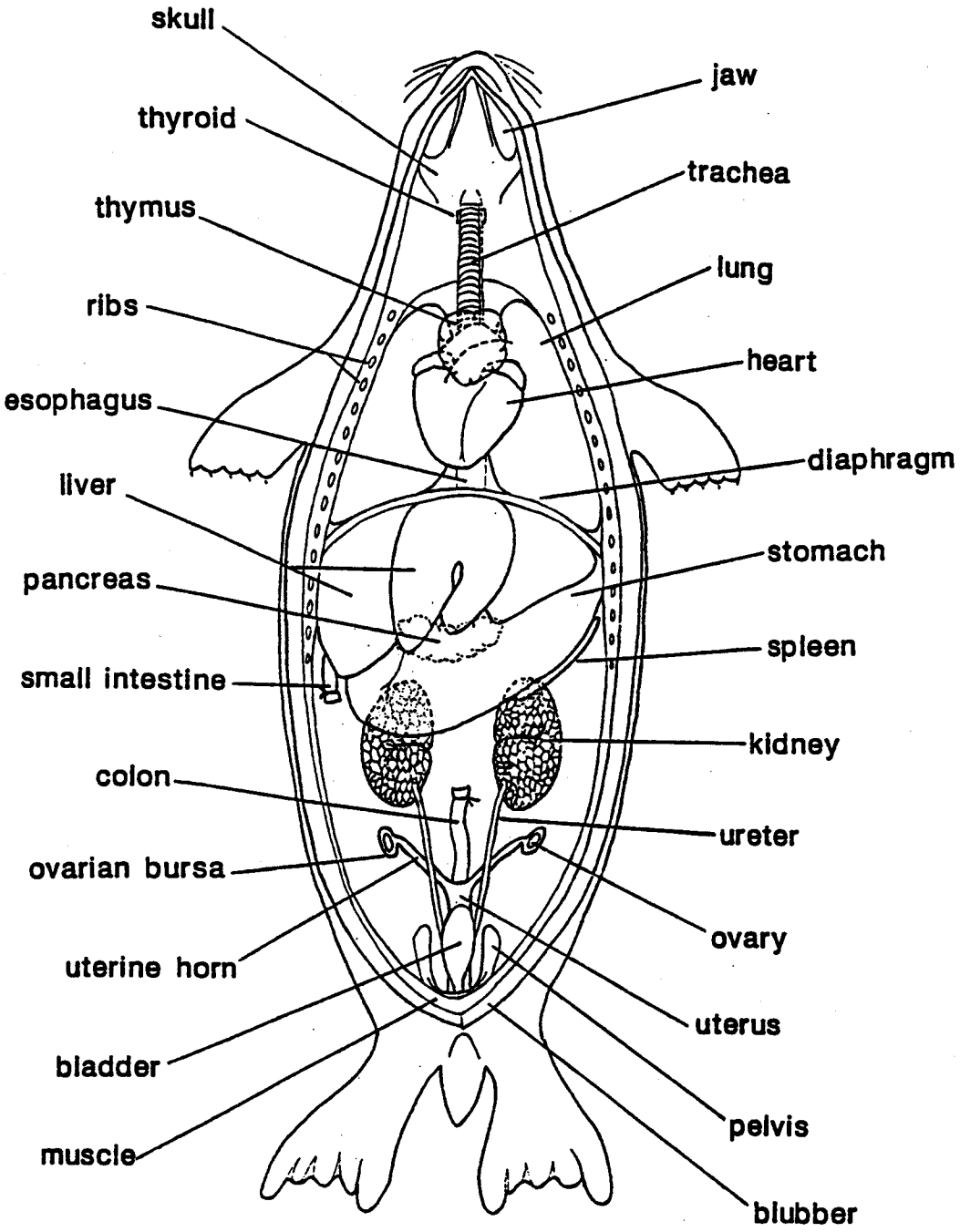


Figure 4.--Some of the internal features of a juvenile female *Monachus schauinslandi*; the intestines and rib cage have been removed, and the diaphragm trimmed to the midline. Note that the broken lines indicate those structures (or portions of structures) obscured by other parts.

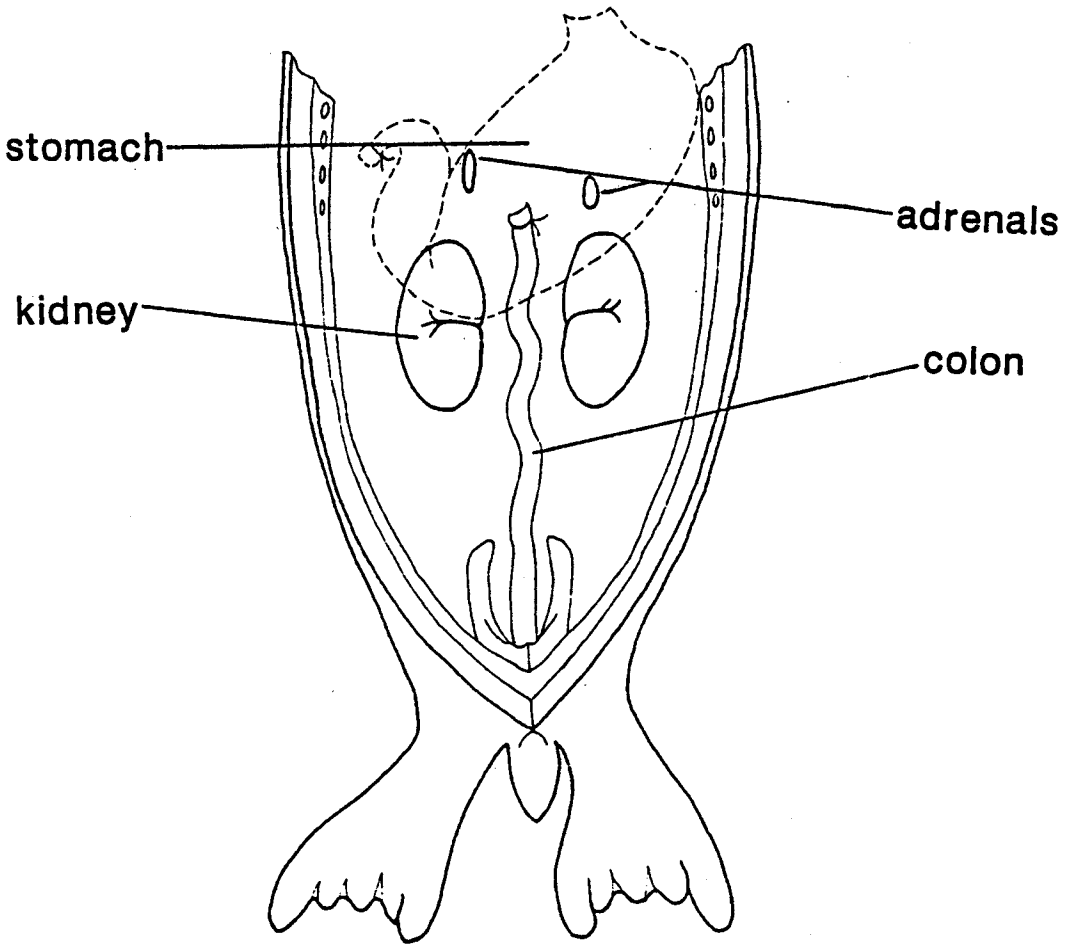


Figure 5.--The placement of the adrenal glands in relation to the kidneys and the stomach in a *Monachus schauinslandi*.

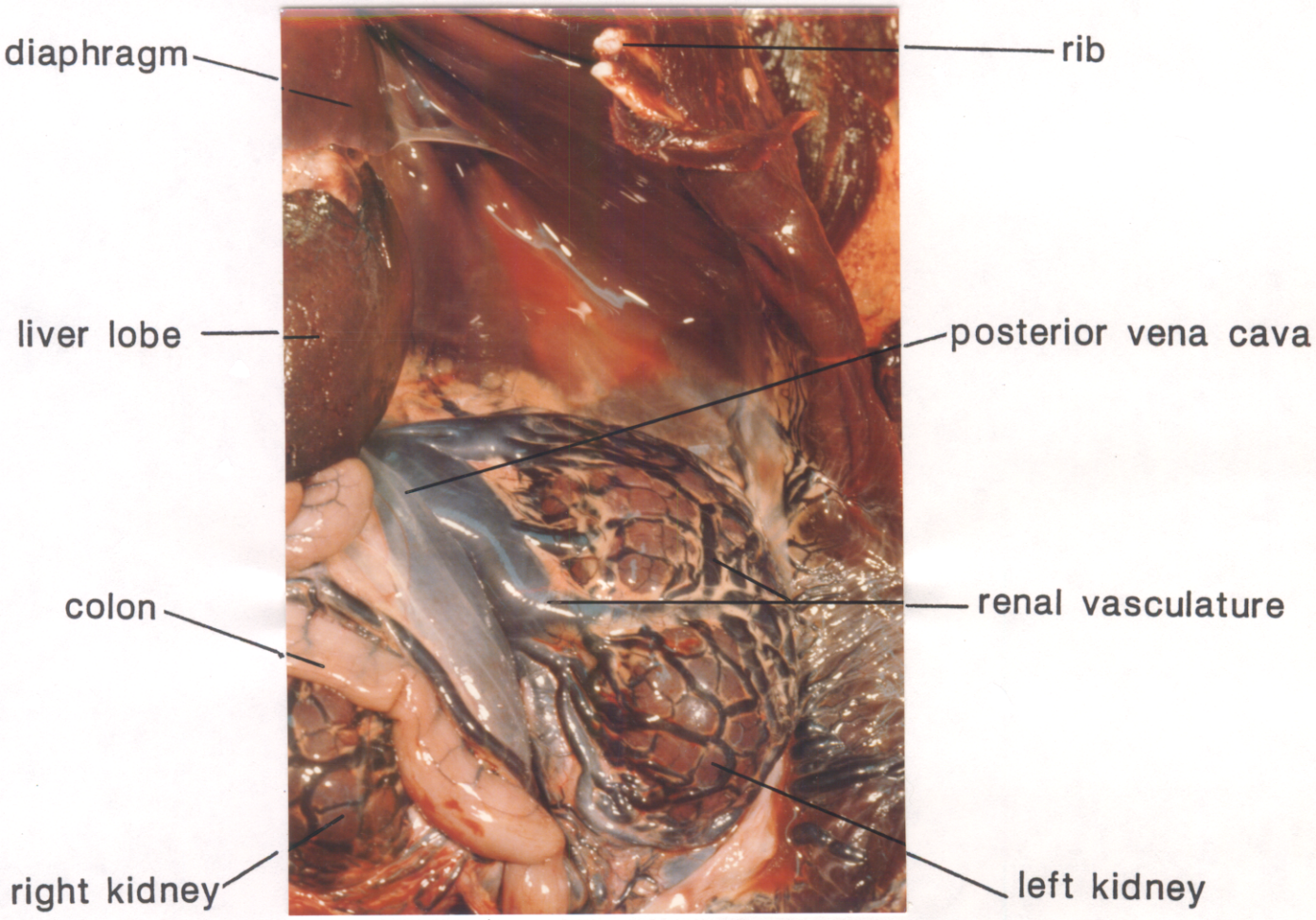


Figure 6.--Ventral aspect of the kidneys in a *Phoca vitulina* with engorged vasculature.

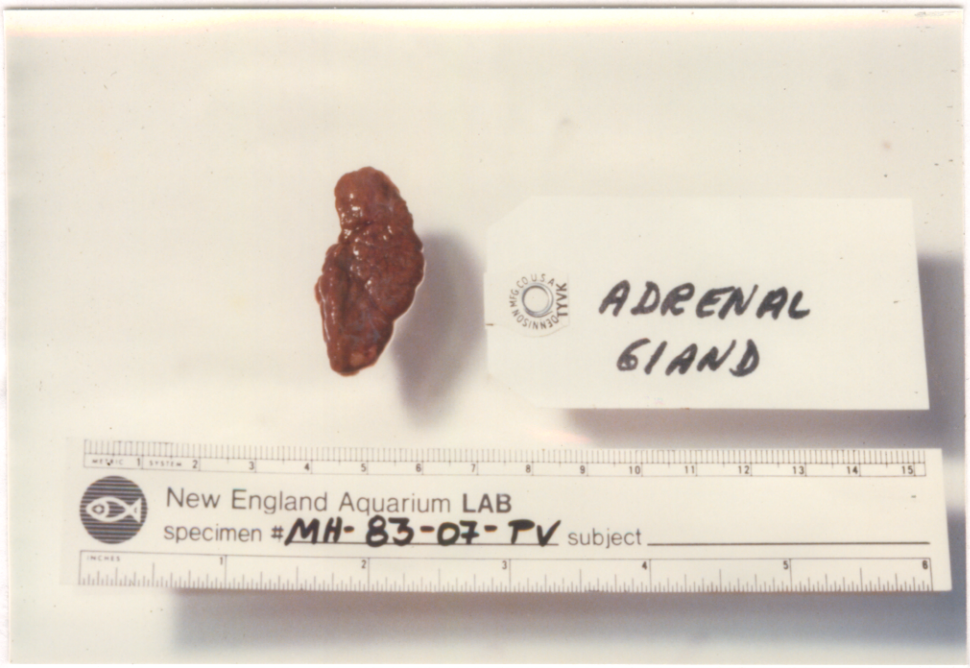


Figure 7.--Adrenal gland from a *Phoca vitulina*.

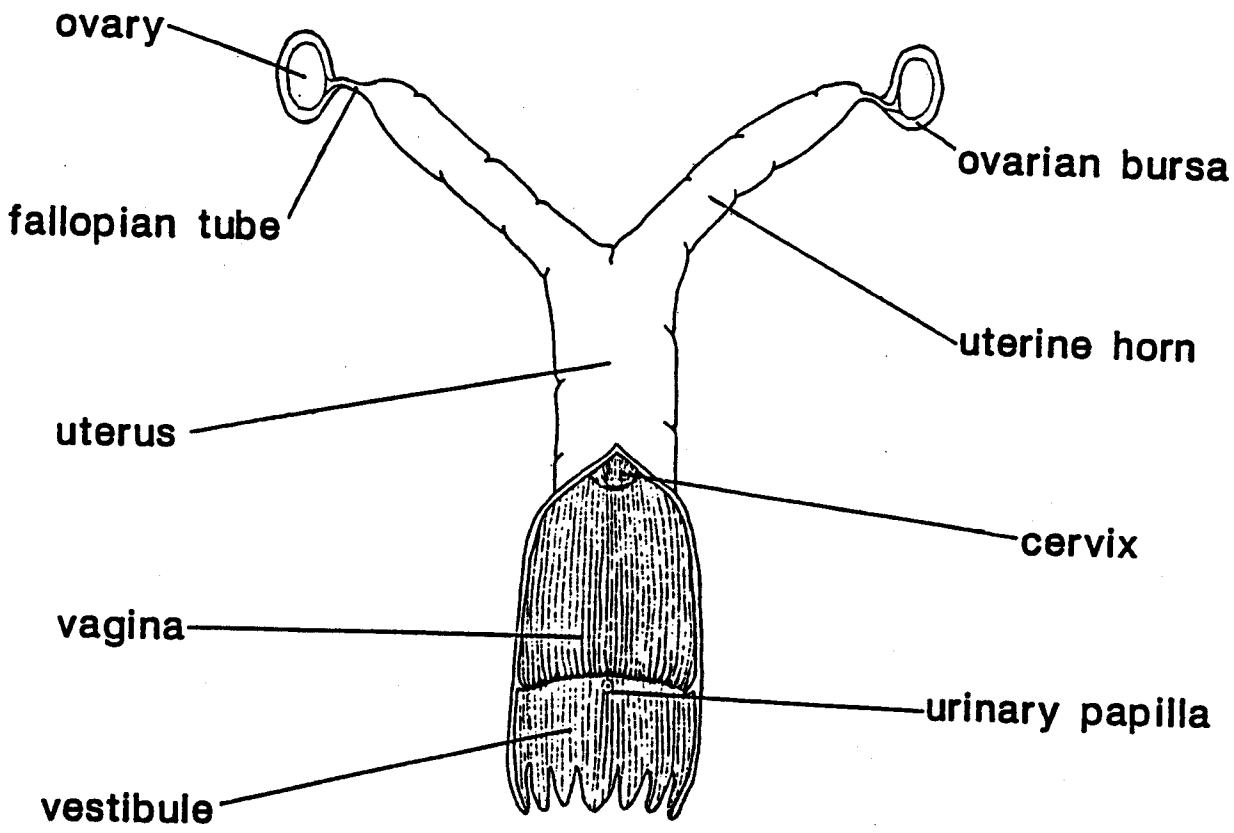


Figure 8.--The female reproductive system from a juvenile phocid with the cervix, vagina, and vestibule revealed.

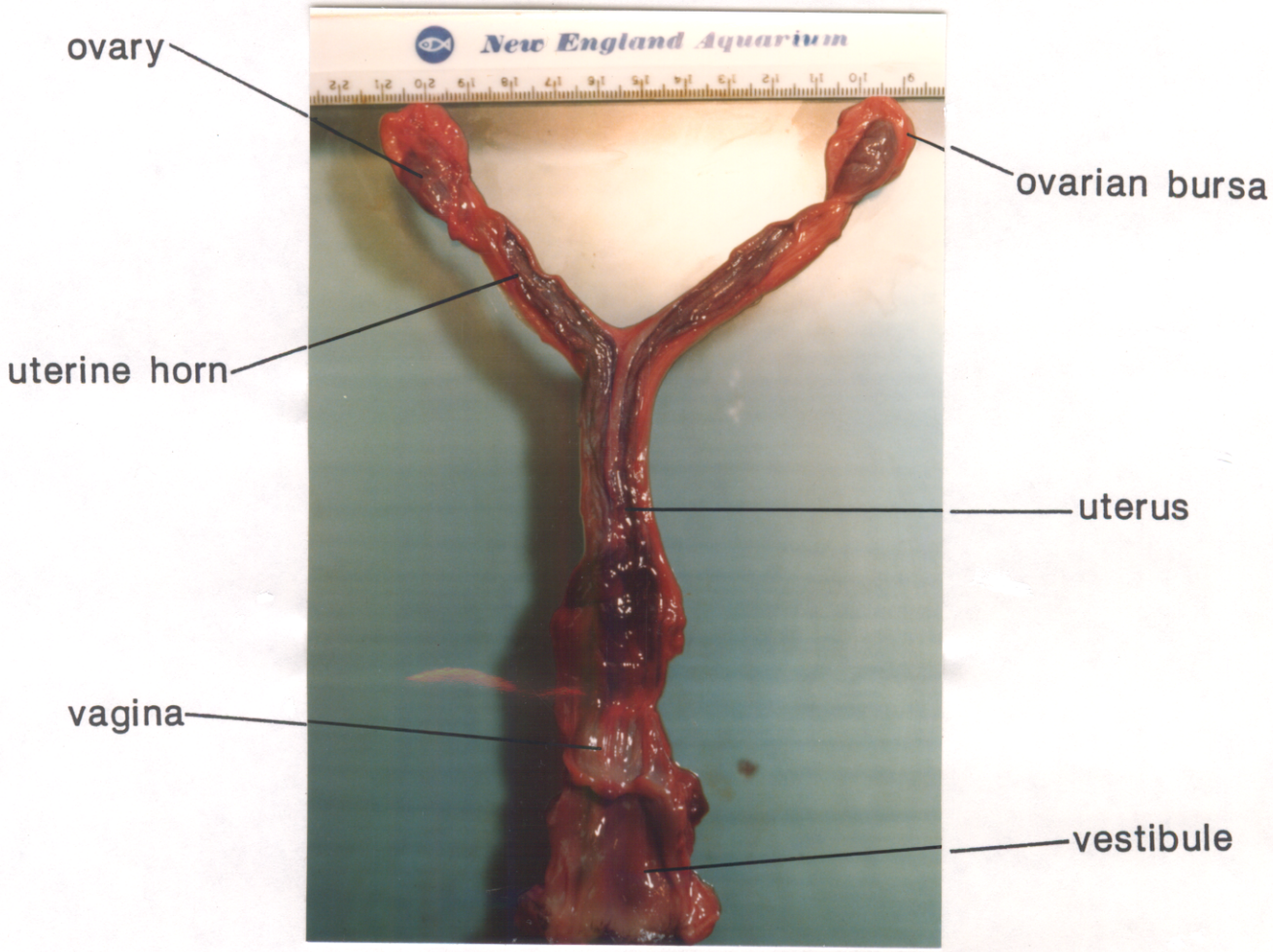


Figure 9.--Dissected female reproductive tract from a juvenile *Phoca vitulina*.

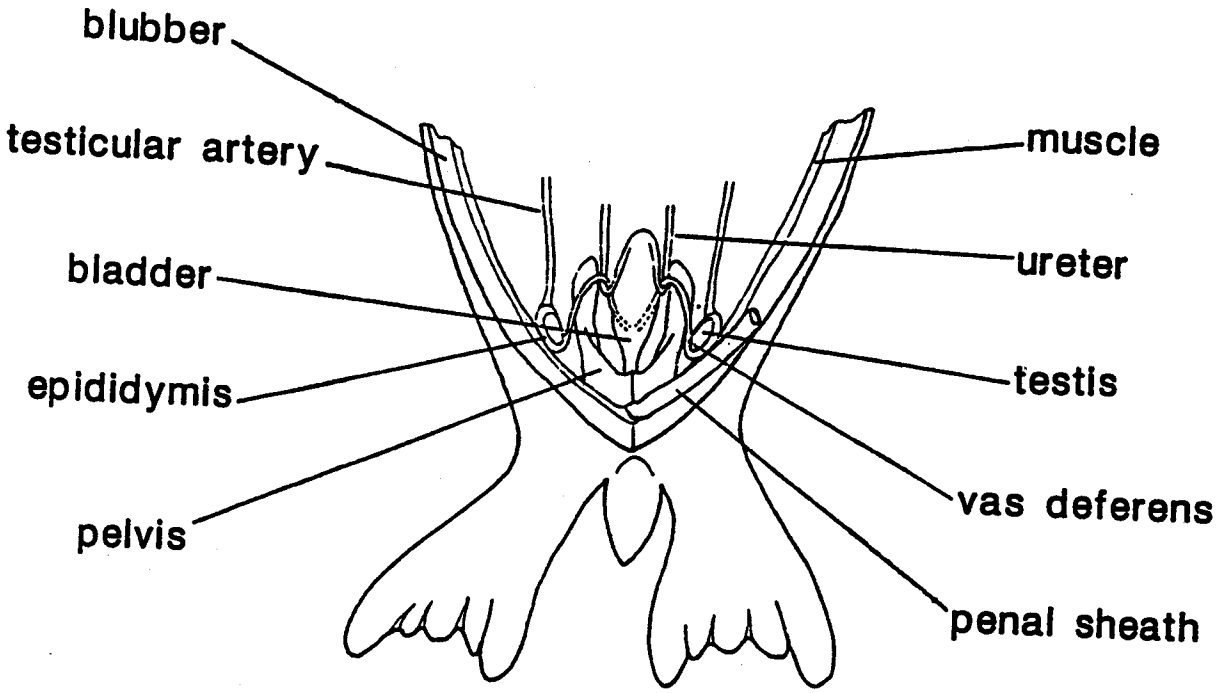


Figure 10.--The reproductive organs in a juvenile male *Monachus schauinslandi*.

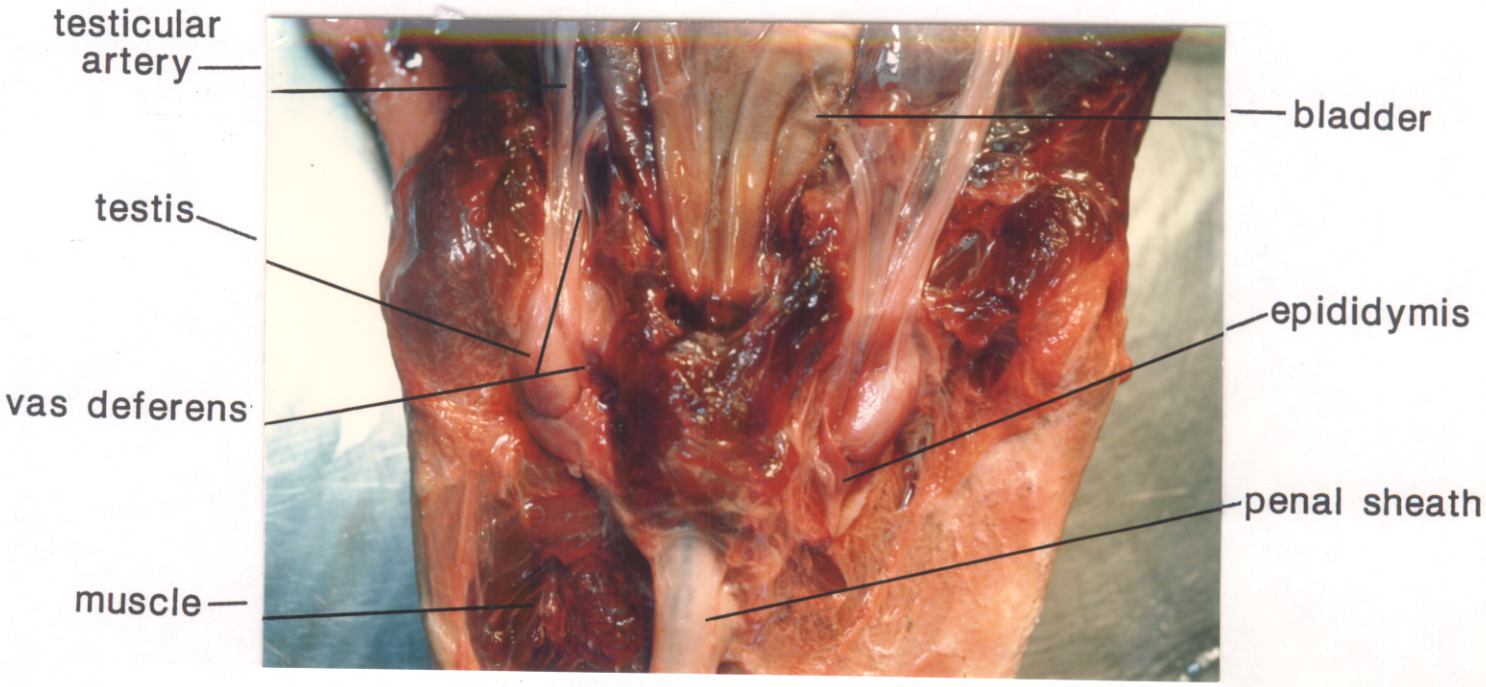


Figure 11.--Ventral aspect of the male reproductive tract in a juvenile *Monachus schauinslandi*.


Figure 12.--Lateral aspect of the spleen from a *Monachus schauinslandi*.

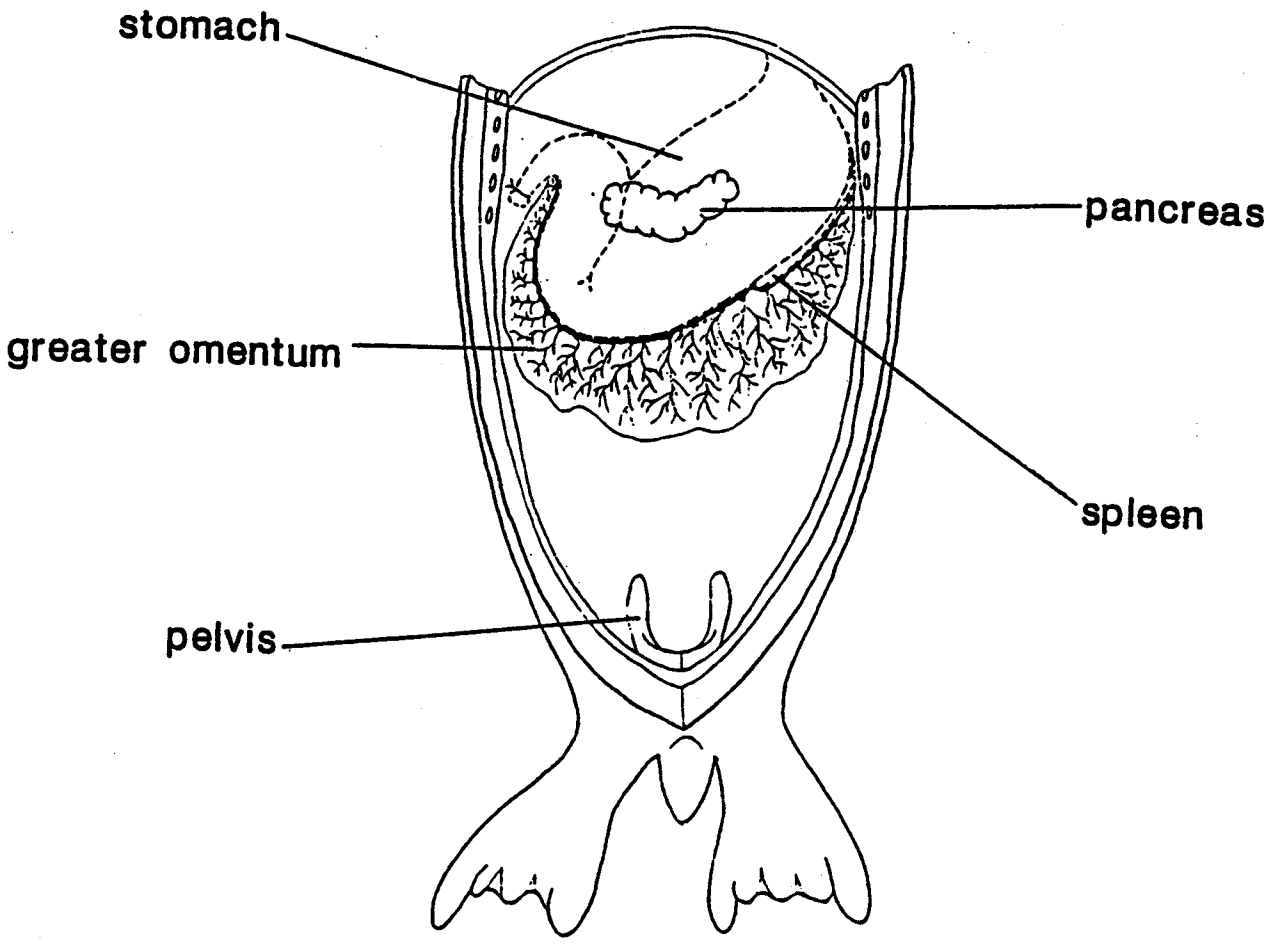


Figure 13.--The placement of the pancreas and greater omentum in relation to the stomach and spleen.

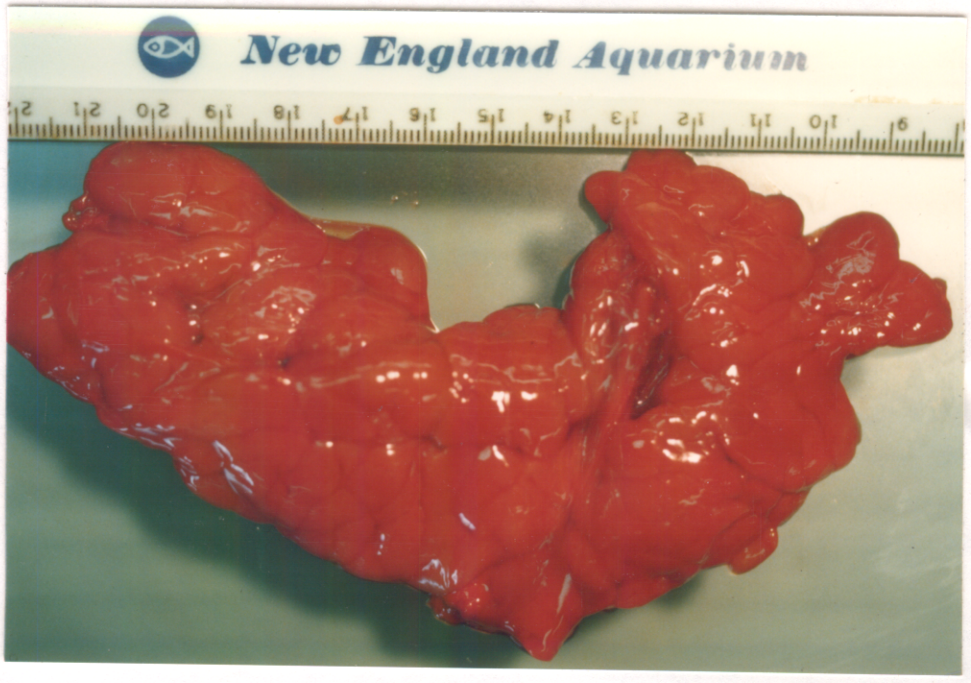


Figure 14.--Ventral aspect of the pancreas from a *Monachus schauinslandi*.

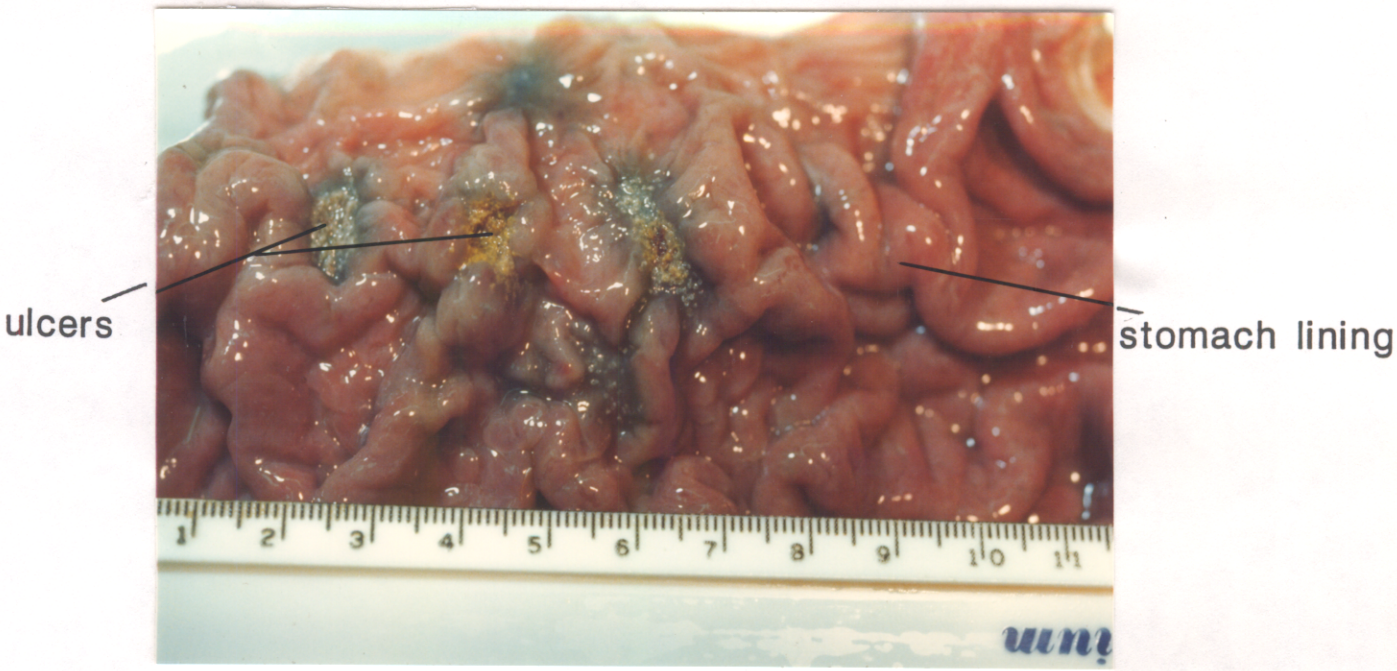


Figure 15.--A portion of an ulcerated stomach lining from a *Monachus schauinslandi*.

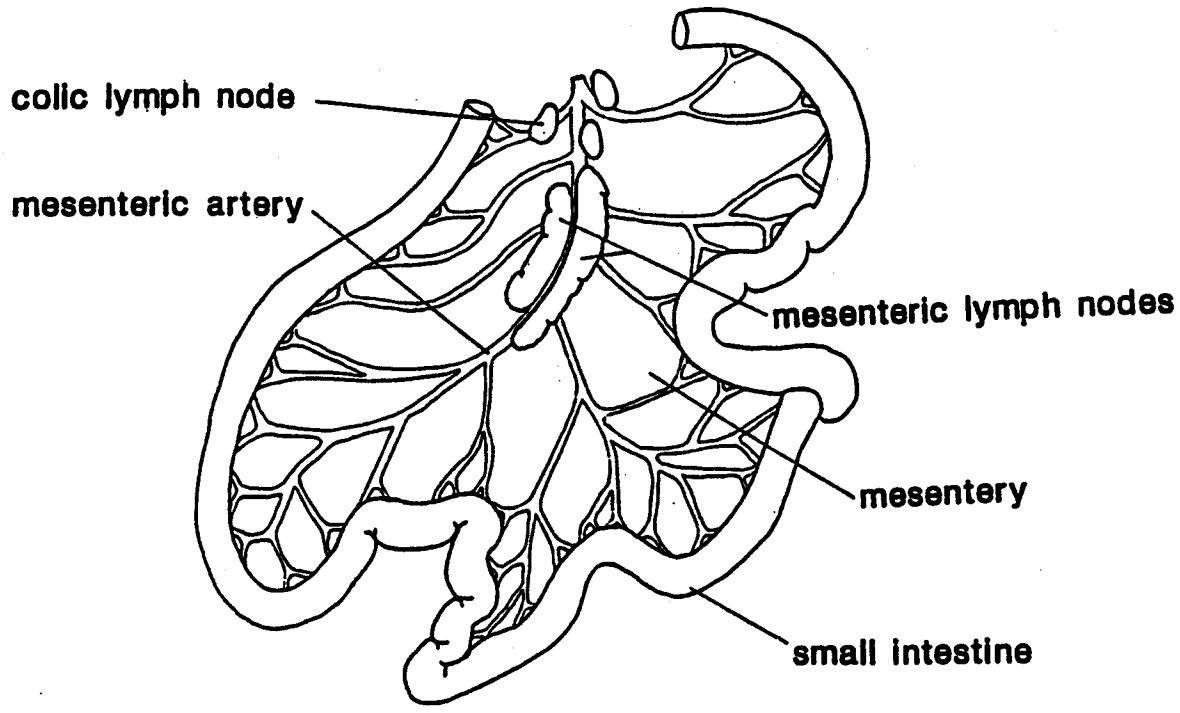


Figure 16.–The mesenteric lymph nodes in relation to a section of the small intestine.

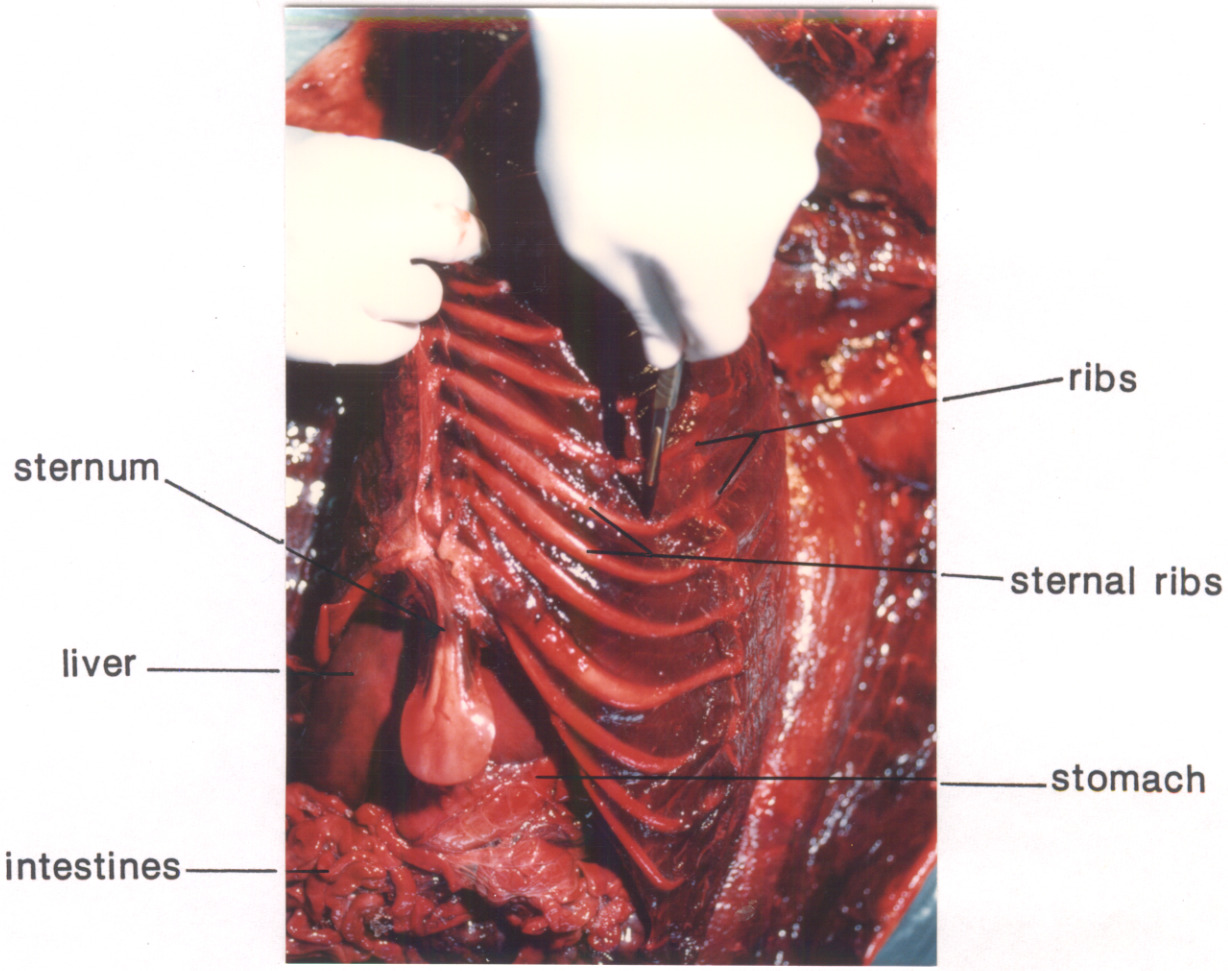


Figure 17.--A technique for removing the ventral aspect of a phocid rib cage.

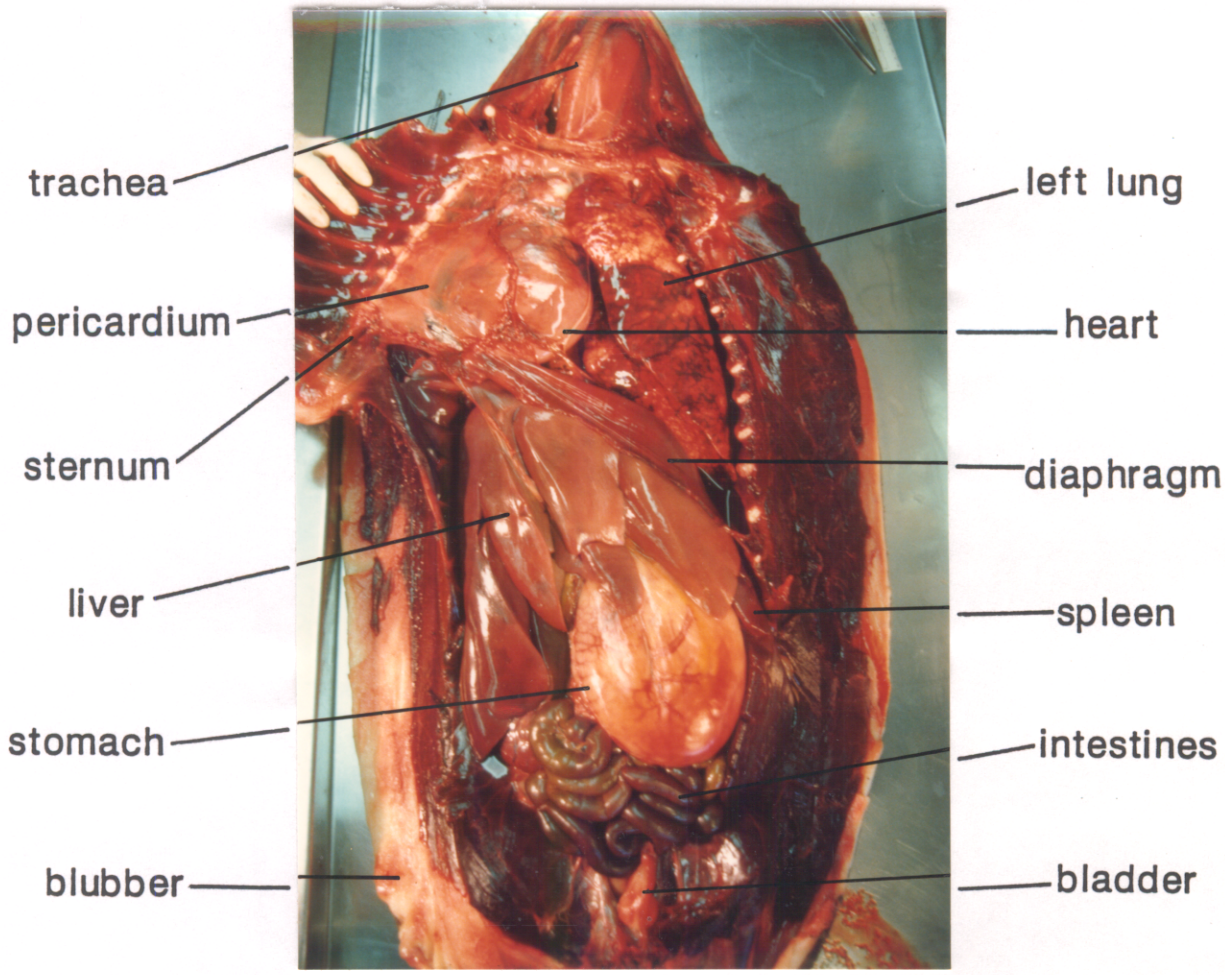


Figure 18.--Ventral aspect of a *Phoca vitulina* with the rib cage cut down one side and pulled over to reveal the thoracic cavity. Abdominal organs are still in place.

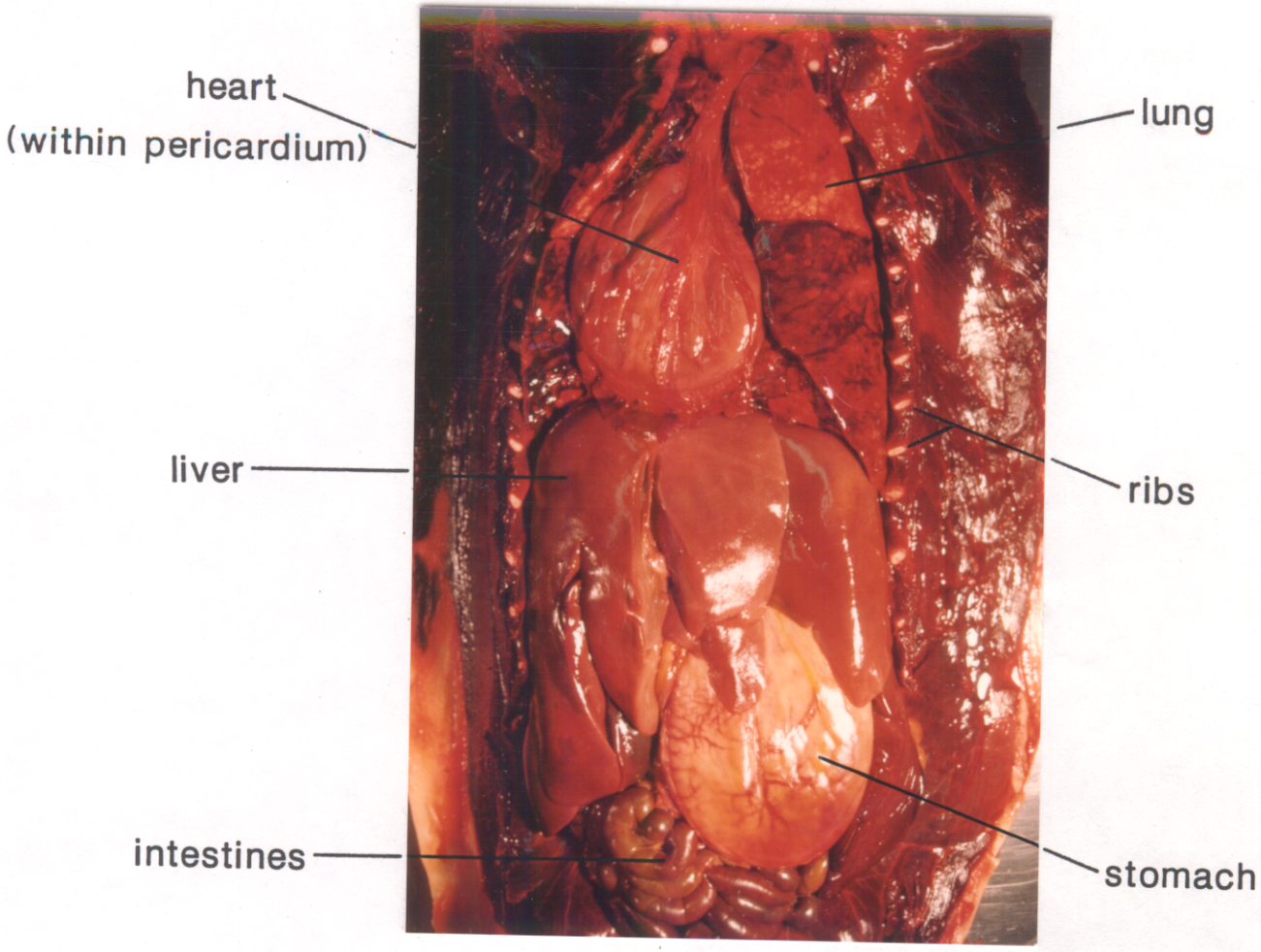


Figure 19.--Ventral aspect of a *Phoca vitulina* displaying the thoracic and abdominal cavities. Abdominal organs are still in place.

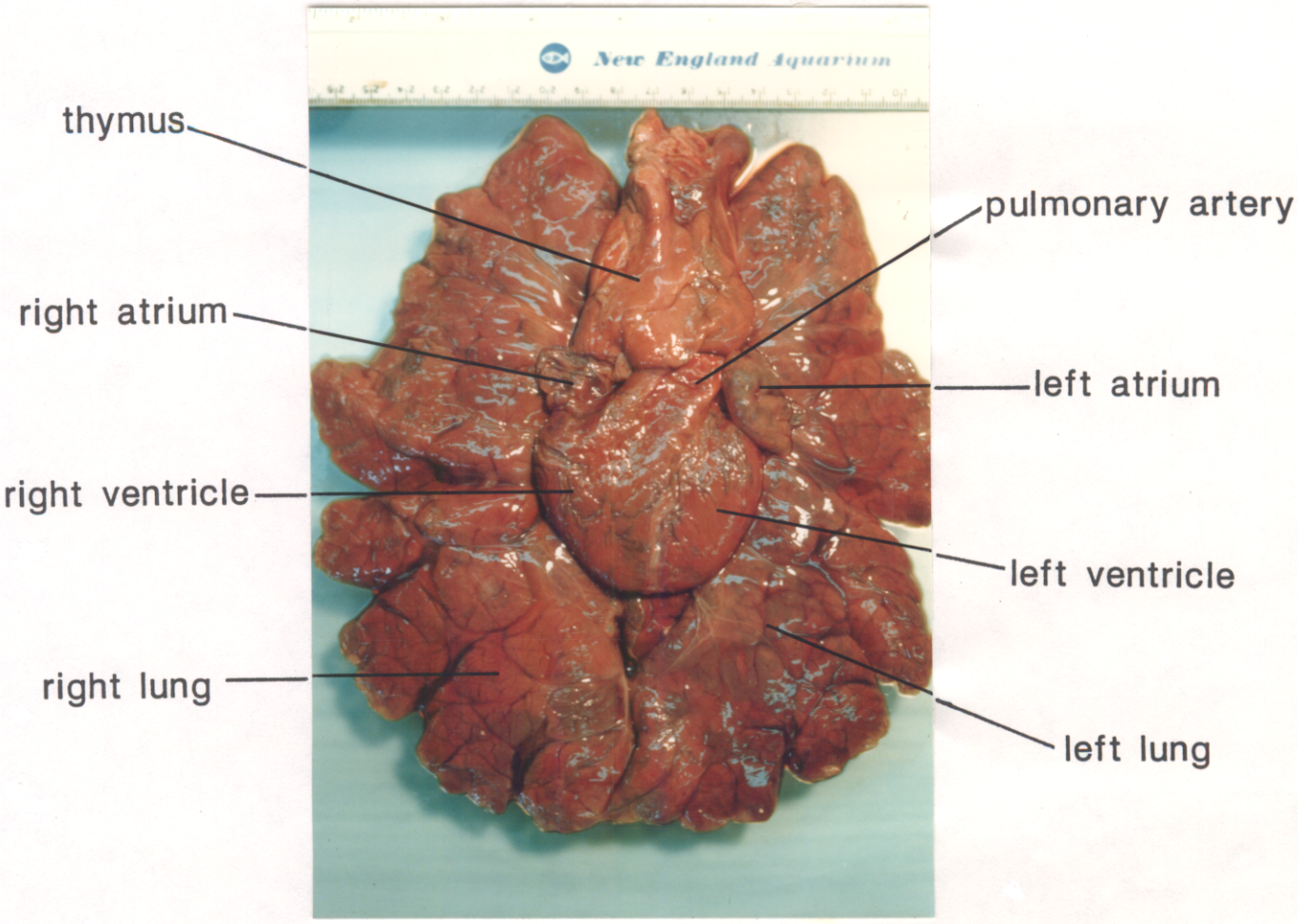


Figure 20.--Ventral aspect of the heart, lungs, and thymus from a stillborn *Monachus schauinslandi*. Note that the coloration of this specimen is atypical (i.e., specimen is pale because the carcass was not fresh when sampling was done).

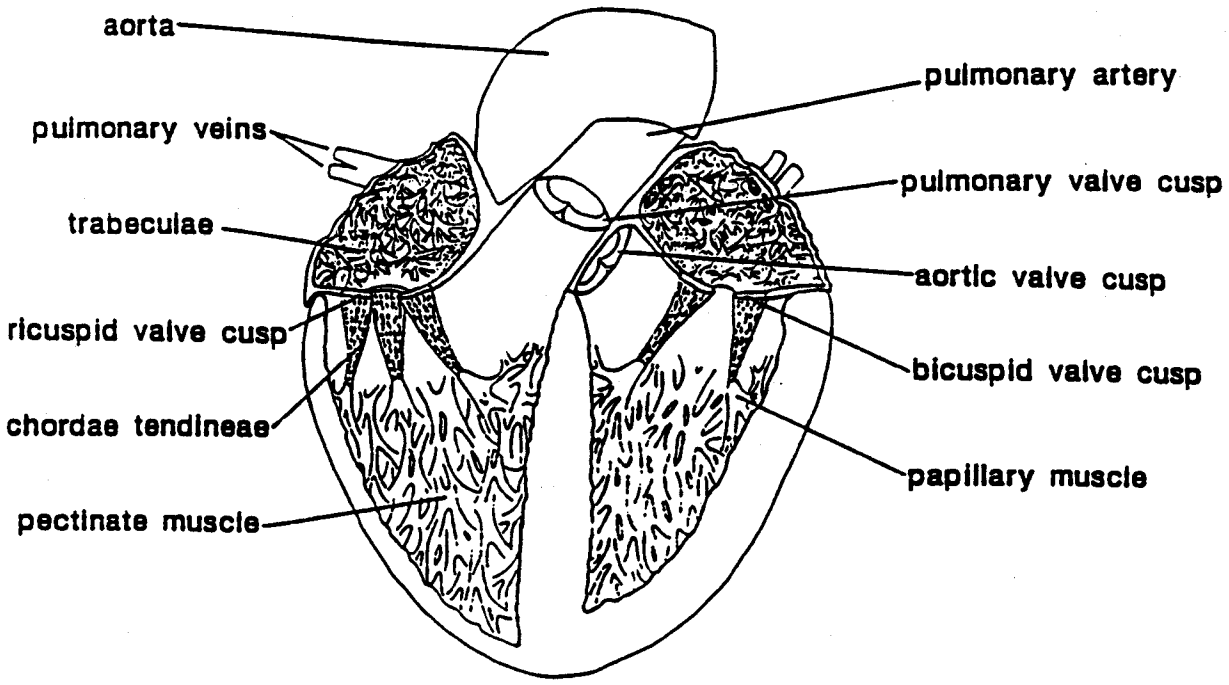


Figure 21.--Ventral aspect of a phocid heart with both atria and ventricles cut open.

# Appendixes

Appendix A.--A quick reference for the preservation techniques and formulae specified in the necropsy manual.

## Fixing Tissues in Formalin

(1) Use a clean scalpel to cut sections 0.5 cm thick (with cut surfaces parallel) and broad enough to orient and identify the origin (2-3 cm across). The sample should contain both diseased and adjacent normal portions of the organ when applicable. If collecting a larger sample of tissue is necessary, make slices across the sample at 0.5 cm intervals, then proceed with the usual fixation steps.

(2) Place tissues in 10% formalin or buffered 10% formalin (the addition of buffers eliminates the formation of undesirable acid-formalin-hematin-pigment in tissue sections). *The volume of formalin should be about 10 times that of the tissue.*

(3) Include two labels with each specimen to identify the tissue and the field number of the specimen. Double-bagging, with one label in the outer bag, is preferable to both labels being placed in the inner bag. Use waterproof tags and a permanent marker for labeling.

(4) After the tissues have been in formalin for 24-48 hours, pour off the formalin and rinse the tissues in fresh water. Replace the specimens and labels in a new solution containing 10% formalin. Staining results are greatly improved by performing this last step. The following tissues should be collected:

Thyroid
Adrenals
Gonad
Kidney
Spleen
Pancreas
Stomach (only if an abnormality is present)
Intestines (only if an abnormality is present)
Mesenteric lymph nodes
Liver
Thymus
Heart
Lung
Brain (only from fresh specimens)

Appendix A.--Continued.

## Preserving Parasites

Preserve ectoparasites in 70% ethanol.

There is no best method that can be recommended for preserving endoparasites. In general, FAA is considered a good fixative for endoparasites. If the parasites are still alive when found in the animal, place them in warm water to relax them, then transfer them to the FAA. Remember to include a label stating the organ with which the parasites were associated and the field number of the specimen.

Check for the following parasites:

Nasal mites (Arachnida)
Pancreatic flukes (Trematoda)
Stomach worms (Nematoda)
Liver flukes (Trematoda)
Intestinal parasites (Cestoda and Acanthocephala)
Heartworms (Nematoda)
Lungworms (Nematoda)

## Preserving Stomach Contents

Stomach contents preserve very well in 70% ethanol. They may also be frozen if the facilities are available.

## Toxicological Assays

Tissue samples of certain organs and blubber are collected for heavy metal or hydrocarbon assays. These samples must be stored frozen, so do not collect them if refrigeration is not available.

Samples of 100 g are collected from each of the organs and tissues listed below. If the samples collected are for heavy metal assay, they may be stored in Whirl-pak bags. However, tissues collected for hydrocarbon assay must not come in contact with any plastic or other petroleum product. Hence, they should be wrapped in nonlubricated, commercial grade aluminum foil, well sealed to keep out contaminants. Store the samples at -20°C or below.

Appendix A.--Continued.

Collect the following samples:

Blubber
Muscle
Kidney
Liver
Brain

## Fixatives and Preservatives

10% buffered formalin

| | |
|---|---|
| Commercial formaldehyde (37-40%) | 100 ml |
| Water | 800 ml |
| Seawater (buffer) | 100 ml |

FAA (alcohol-acetic-acid-formaldehyde-mixture)

| | |
|---|---|
| Ethanol (70%) | 900 ml |
| Acetic acid, glacial | 50 ml |
| Commercial formaldehyde (37-40%) 50 ml | |

70% ethanol

| | |
|---|---|
| Ethyl alcohol (95%) | 735 ml |
| Water | 265 ml |

Appendix B.--Special collections.

The following collections are only made under special circumstances. They are included in the manual for the rare instance when a stranded pinniped is found either dying or very recently dead (died within the last few hours) and laboratory facilities (or at least refrigeration) are also accessible. These samples are quite valuable diagnostically, so every effort should be made to take them, when possible. They are virtually worthless if the animal has been dead for more than a few hours or if refrigeration is unavailable.

## Bacterial and Viral Samples

This procedure is the isolation of samples suspected of containing bacterial or viral agents responsible for a given condition present in the animal. Aseptic technique is essential for both bacterial and viral isolates. Instruments should be sterile, and organs from which a microbiological sample is to be taken must be exposed carefully to avoid external contamination. If the surface of the affected organ has already been contaminated, the area where the incision is to be made should be swabbed with 10% formalin or seared with a red hot scalpel. Allow the formalin to air dry before proceeding with the incision. Use a sterile scalpel to make the incision.

For bacterial isolates, dip a sterile culturette swab into the incision, twirling it slightly against the cut surfaces of the lesion, then immerse it into the culturette vial containing the transport medium. Break off the stick below the part that has been handled. Cap, seal, and label the vial, then refrigerate it (do not freeze).

For viral isolates, collect a 5 x 5 x 10 mm sample of tissue, aseptically; place it in a Whirl-pak bag, label it, and store frozen (-20°C or below). Note the isolates collected on the necropsy form.

## Collecting Blood

Take the blood immediately after the thoracic cavity has been exposed. Use a sterile hypodermic needle and syringe to collect a sample of blood from the right ventricle. First slice through the pericardium in order to reveal the heart. Then insert the needle into the ventricle and slowly draw the blood from this chamber. Note on the necropsy form that blood was collected.

Blood samples from which serum is to be collected should be handled very carefully and protected from freezing to prevent hemolysis. After the blood sample has been collected, centrifuge and remove serum or let the sample stand at room temperature for 12 to 24 hours, during which a clot will form and retract. Then draw or decant the serum into clean, sterile containers, preferably in 1-3 ml subsamples, then freeze it. Avoid repeated thawing and refreezing of these samples.

Appendix B.--Continued.

## Special Equipment

Syringes
Hypodermic needles (18 g)
Blood collecting vials containing E.D.T.A.
Sterile vials for serum

# GLOSSARY

# GLOSSARY

**Abdomen** - the portion of the body cavity that lies between the diaphragm and the pelvis and also encloses the viscera

**Abscess** - a localized collection of pus surrounded by an inflamed area

**Acanthocephala** - thorny headed worms

**Adhesion** - an abnormal fusing of an organ or part, with the structure adjacent to it

**Arachnids** - mites and ticks

**Aseptic** - sterile; devoid of contaminants

**Atrium (pl. atria)** - a chamber of the heart for collecting blood

**Axilla** - the armpit

**Bile** - the brownish-yellow or greenish-yellow liquid that is secreted by the liver, stored in the gall bladder, and emptied into the duodenum

**Blubber** - the layer of fatty tissue between the skin and muscle layers

**Broad ligament** - paired double folds of the peritoneum which attach to the dorsolateral walls of the abdominal cavity and contain the ovaries, oviducts, and uterine horns

**Bronchus (pl. bronchi)** - either of the main divisions of the trachea, each leading to a lung

**Calyx** - the funnel-shaped collecting duct of a reniculus

**Capsule** - the fibrous or membranous sheath encasing an organ or part

**Caudal** - of, relating to, or located near the tail or hind end of the body

**Cestoda** - tapeworms

**Chordae tendineae** - fibromuscular cords that arise from the walls of the ventricle and attach to the free borders of the tricuspid and mitral valve cusps

**Clot** - a coagulated mass or lump of fibrin and blood cells that forms when the blood congeals

**Congestion** - an excessive accumulation of blood within an organ

**Copious** - abundant

**Corpus albicans (pl. corpora albicantia)** - the scar left on an ovary from the degeneration of a corpus luteum

**Corpus luteum (pl. corpora lutea)** - a yellow glandular swelling in the ovary formed by the cells of an ovarian follicle that has matured and discharged its ovum

**Cortex** - the outer layer of an organ or part, as of the kidney or adrenal

**Cyst** - a fibrous, membranous sac within a tissue or organ containing a gaseous, liquid, or semisolid substance

**Deflect** - to turn aside

**Dorsal** - of, toward, on, in, or near the back

**Ectoparasite** - a parasite that lives on the exterior of an organism

**Endoparasite** - a parasite that lives parasitically within another organism

**Fibrin tags** - tags formed of an elastic, insoluble protein derived from the interaction of fibrinogen with thrombin

**Fibrotic** - tissue of a fibrous nature, as found associated with certain reparative or reactive processes, in excess of amounts normally present

**Flaccid** - lacking firmness; flabby

**Foramen magnum** - the opening in the skull for the spinal cord and associated structures

**Foramen ovale** - a perforation in the wall between the two atria present in fetal pinnipeds

**Fore** - located at or toward the front; anterior

**Hepatic sinus** - a venous sinous formed from the enlarged hepatic veins and posterior vena cava; located just posterior to the diaphragm

**Hemolysis** - rupturing of the red blood cells due to damage or breakdown of the cell walls

**Hemorrhage** - a large discharge of blood from the blood vessels

**Infarct** - a pale and necrotic region of tissue resulting from failure of the local blood supply

**Inferior** - situated under or beneath

**Inguinal** - of, relating to, or located in the region of the groin

**Lateral** - of, relating to, or situated at or on the side(s)

**Lesion** - an abnormal structural alteration due to injury or disease

**Lumen** - the inner open space of a tubular organ

**Medial** - relating to, situated in, or extending toward the middle

**Medulla** - the inner core of certain anatomical structures such as the adrenal and kidney

**Mesenteries** - the translucent membranous tissue that connects the intestines to the dorsal abdominal wall

**Nares** - the openings of the nasal cavities; nostrils

**Nasopharynx** - the area of the pharynx immediately behind the nasal cavity and above the soft palate

**Nematoda** - worms having unsegmented, threadlike bodies

**Otolith** - one of the calcareous particles found in the inner ear of fishes (and some other vertebrates) used for age determination

**Oviduct** - the narrow tube which carries the eggs from the ovary to the uterine horn

**Palpate** - to examine or explore by touching (an organ or area of the body) as a diagnostic tool

**Papillary muscle** - the conical-shaped muscular projections which give rise to the chordae tendineae

**Pathogenic** - capable of causing disease

**Pectinate muscle** - the interlacing muscular bands which line the walls of the atria

**Perforation** - a hole or series of holes punched or bored through something

**Pericardium** - the fibroserous envelope of the heart

**Peritoneum** - the membranes lining the walls of the abdominal cavity and enclosing the viscera

**Pharynx** - the section of the digestive tract that extends from the nasal cavities to the larynx, there becoming continuous with the esophagus

**Plaque** - a small disk shaped growth or patch

**Pleura** - the membrane which lines each half of the thorax and is reflected over the surface of each lung on the same side

**Post mortem** - occurring, done, made or formed, after death

**Post partum** - of or occurring in the period shortly after birth

**Posterior** - located behind a part or toward the rear of a structure

**Pubic symphysis** - the union of the two halves of the pelvis on its ventral aspect

**Pulmonary** - pertaining to the lungs

**Remnant** - the remainder; that which remains of a thing after a part has been removed

**Reniculus (pl. reniculi)** - the units that comprise the pinniped kidney Each reniculus functions as a separate unit containing its own cortex, medulla, and calyx

**"Seal finger"** - an acute bacterial disease marked by fever and severe skin inflammation

**Semilunar valves** - three strong semicircular pocketlike folds of the lining of each ventricle which prevent blood from flowing back from the pulmonary artery and aorta

**Serum** - the watery portion of an animal fluid remaining after coagulation

**Sever** - to separate or part

**Thoracic cavity** - the cavity in the body between the neck and the abdomen in which the heart, lungs, esophagus, etc, are situated

**Torso** - the trunk of the body

**Trematoda** - flukes and their allies; all are parasitic

**Tubercular** - of, relating to, or covered with tubercules (nodules) characteristic of tuberculosis

**Tumor** - an abnormal mass of tissue, not inflammatory, and independent in character

**Ulcer** - an eroding or festering sore

**Vena cava** - one of the large veins by which blood is returned to the right atrium of the heart. The posterior vena cava returns blood from the posterior parts of the body and viscera

**Ventral** - pertaining to the lower side or surface of an organ or structure

**Vestibule** - the space between the labia minora containing the orifice of the urethra

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